Vol. III, No. 4 April, 1917

THE ANNALS OF APPLIED BIOLOGY

THE OFFICIAL ORGAN OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

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The Association of Economic Biologists

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VOLUME III APRIL, 1917 No. 4

ACCESSORY WETTING SUBSTANCES WITH SPECIAL REFERENCE TO PARAFFIN EMULSIONS.

BY A. H. LEES, M.A.,

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WITHIN the last three years or so the opinion has been gradually gaining ground that one of the most important points in a spray fluid whether for fungicidal or insecticidal purposes is its wetting power. The author's attention was first called to this point when using certain proprietory insecticides which, used according to the directions given, were not very effective but which, on addition of soft soap, had their wetting powers and therefore their killing powers greatly increased. Reference was made to this subject in the *Annual Report for* 1913 *of the Research Station, Long Ashton*[1], and since that time other papers have appeared dealing more fully with the physical problems involved in the question[2,3]. It is not proposed here to touch this side of the problem as the author is not a physicist but to give certain results bearing more on the immediately practical aspects of spraying.

It has long been known that the addition of soft soap to a fluid containing no substance capable of precipitating soap, causes an increase of wetting power, No quantity of soap, however, has proved sufficient to wet such resistant insects as the colonies of Woolly Aphis which occur on apple trees. These insects excrete a covering of waxy threads on their back which render them impervious to ordinary spray fluids. The same difficulty was found in the experiments of Prof. Barker and the author against American Gooseberry Mildew. There was therefore a real need to find a satisfactory accessory wetting agent.

[1] *The Annual Report of the Agric. and Hort. Research Sta., Long Ashton*, Bristol, p. 70.

[2] Lefroy, "Insecticides." *Ann. App. Biol.* Jan. 1915.

[3] W. F. Cooper and W. H. Nuttall, "The Theory of Wetting and the Determination of the Wetting Power of Dipping and Spraying Fluids containing a Soap Basis." *Journ. Agric. Sci.* Vol. VII, pt. 2, Sept. 1915.

In this connection paraffin emulsions were tried. These emulsions have been often recommended by many writers as insecticides and there is no doubt that they are of value. They have, however, a reputation of being dangerous to use owing to their liability to scorch foliage. There are, the author believes, very definite reasons for this liability but by suitable means it may be got rid of.

An emulsion may be stable or unstable, its state depending on its constitution. If stable, every small paraffin globule is surrounded by a film of soap and the film is sufficiently strong to keep one globule from touching and coalescing with its neighbour. If very little soap is originally present, or if where present in sufficient quantities it is withdrawn by chemical means, there comes a point where the film is no longer strong enough to prevent paraffin globules from touching and fusing with their neighbours. When this happens throughout the emulsion, paraffin appears in quantity on the surface and the emulsion is said to be unstable.

Pickering[1] has pointed out the great diversity of formulae for paraffin emulsions that have been recommended. In a table given by him the proportion of paraffin to soap in one formula is as great as 100 : 1·2, in others as low as 100 : 240, while there are all kinds of intermediates. This enormous range in quantities suggests that some of these formulae have been advocated in a somewhat haphazard spirit, since if formulae at one end of the series were successful it does not seem necessary to make use of others which contain such divergent quantities.

Of the 24 formulae quoted 15 contained paraffin in proportion to soap in ratios varying from 100 : 23 up to 100 : 1·2. That is to say in those mixtures of these fifteen where the paraffin bore the lowest ratio to the soap, it was present to the extent of four times the amount of soap and in those which bore the highest ratio, to as much as eighty-three times. It was therefore at first sight probable that the liability to scorching was caused by the high content of paraffin. Experiments were therefore started to see how little paraffin need be used in order to be lethal and at the same time to avoid scorching. It was at once found that with the Bristol water which has a hardness of about 12° it was impossible to obtain any kind of stable emulsion with even small quantities of paraffin unless at least ½ % of soft soap were used. The paraffin simply floated on the surface of the liquid and when sprayed on to foliage naturally produced scorching.

In this connection it is interesting to notice that out of 23 formulae

[1] *6th Report, Woburn Exper. Fruit Farm,* 1906.

quoted by Pickering in which the amount of soap was stated, in 7 it is ·5 % or lower. In these 7 therefore, supposing hard water had been used, however little paraffin were added, scorching would be probable. In the others where the paraffin content was relatively high and the soap content relatively low there would also be a likelihood of scorching since, as will be shown later, the amount of paraffin that can be held in stable emulsion depends largely on the amount of soap present.

Preliminary experiments had shown that it is possible to obtain wetting from paraffin emulsions in two ways. If the ratio paraffin to soap was low, wetting, if it occurred, was due to the aqueous solution, while if the ratio was high, wetting was due to a paraffin film. It was clearly necessary to avoid this wetting by paraffin as to it, moderate scorching, when it occurred, was probably due.

In order to test the properties of paraffin emulsions of different formulae with regard to stability and wetting power a series of experiments was started in the laboratory. The percentages of paraffin tried were, 0, 1, 2, 5, 10, 15, 20 and 25 and the percentages of soap $\frac{1}{2}$, 1, $1\frac{1}{2}$ and 2. Mixtures were made with both distilled and tap water. Each emulsion contained 100 c.c. of water and was made by syringing the mixture of soap and paraffin, without the use of heat, several times through the "rose" of a garden syringe.

Two points were then investigated:

(*a*) The wetting power of the mixture,

(*b*) Its stability.

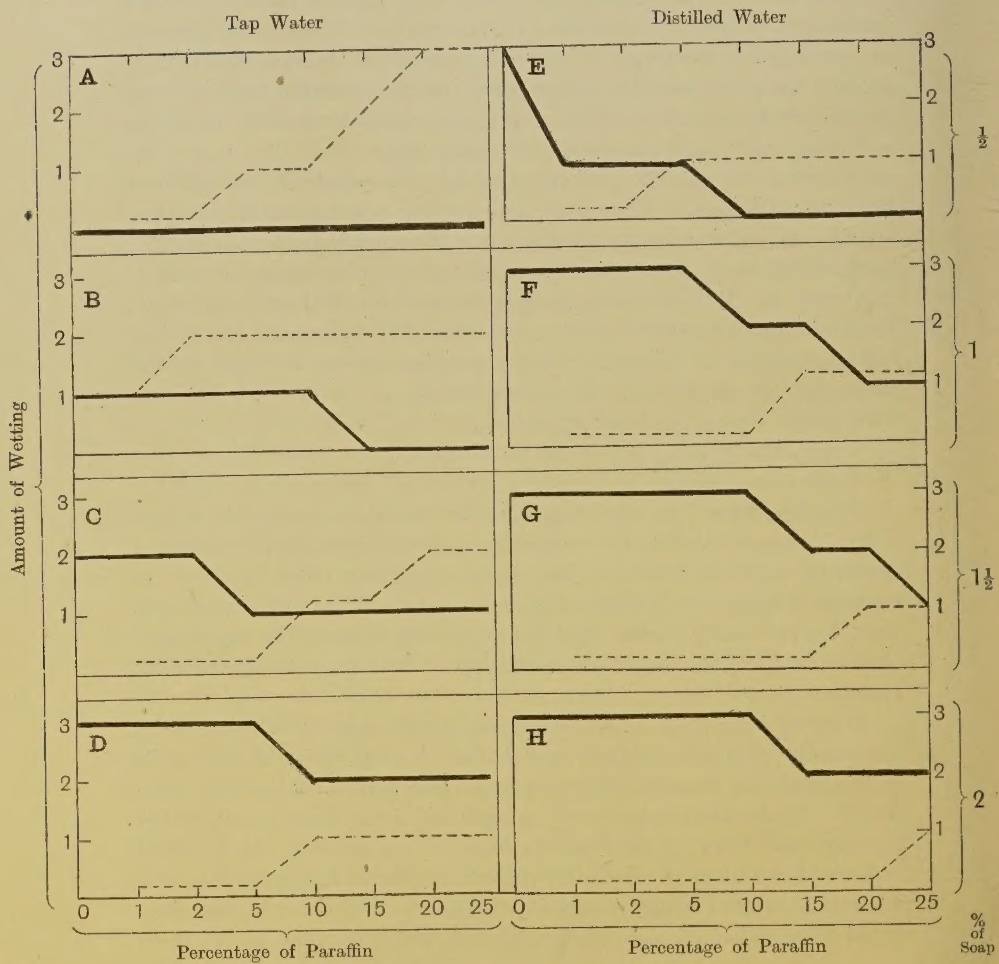
Wetting Power. As these experiments were done during the winter it was necessary to select some surface other than insects or fungi on which to test the wetting. The glossy American cloth cover of an exercise book proved a fairly good substitute. When the surface was worn by the action of the liquids used it was renewed by applying a thin film of boiled linseed oil which, when dry, gave a new brilliant surface.

Wetting took place either by means of the aqueous solution or by the paraffin or by a combination of both. A drop or two of the liquid to be tested was placed on the American cloth surface and gently made to roll. If the drop immediately spread and would form a thin stable aqueous film it was given 3 marks for aqueous wetting. If it spread but would not form an absolutely stable thin film it was given 2 marks, while if it wetted slightly only it was given 1 mark. If the drop failed to spread much and left the faintest film of paraffin behind it on

being made to roll it was given 1 mark for paraffin wetting, if the paraffin mark was definite, 2 marks and if abundant, 3 marks.

In the accompanying table the continuous line represents wetting by water and the dotted line wetting by paraffin. For the sake of clearness, where the dotted line lies along the zero line or along the thick line, it has been raised slightly above.

TABLE I



In considering these plotted results it is probable that as soon as the paraffin wetting rises above zero the mixture would have a slight tendency to scorch and a much greater tendency when wetting by paraffin attained a higher value than wetting by water. Considering the results obtained with tap water first it will be noticed that with only ½ % of soap no wetting by water could be obtained and that with increase of paraffin the wetting by it increased to a maximum. The lower percentages therefore would be unsafe and the higher percentages would certainly produce scorching effects.

With 1 % soap there would be a slight tendency to burn even with only 1 % of paraffin but in practice, owing to the small quantity of paraffin present, this does not occur. With larger quantities such as 5 % and over burning does actually take place. With 1½ % of soap the doubtful point is only reached with 10 % of paraffin and there would be no serious danger until 20 and 25 % was reached. For 2 % of soap probably all strengths would be safe, though for tender foliage there might be some small risk above 5 % of paraffin. With distilled water the doubtful points occur at 5 % for ½ % soap, at 15 % for 1 % soap, at 20 % for 1½ % soap and at 25 % for 2 % soap.

It will be noticed that the ½ % distilled water results are intermediate between the 1 % and 1½ % tap water results. This indicates that in water with a hardness of 12° (such as we have in Bristol) about ¾ % soap is used up in precipitating the metallic salts which cause hardness. That amount of soap is therefore wasted. (This represents about ¾ lb. per 10 gallons and involves a loss of about one penny for that amount of spray fluid.)

From a practical point of view the following recommendations may be drawn from these results. Using tap water of a hardness of 12° or using soft water the amounts of soap to be used for various percentages of paraffin in order to obtain a safe mixture are shown in the following table:—

Table II.

Tap water		Soft water	
% paraffin	% soap required	% paraffin	% soap required
10—25	2	20—25	2
2—10	1½	10—20	1½
1	1	5—10	1
—	—	1—5	½

In cases where the hardness of water is more or less than 12° the greater or less quantity of soap may be approximately calculated by assuming that 12° hardness equals ¾ % soft soap Thus for water of

24° hardness $2\frac{1}{4}$ % soap will be required for a 10 % paraffin emulsion while for only 6° hardness only $1\frac{1}{8}$ % will be required.

Stability of Emulsions. In considering stability of paraffin emulsions it must be pointed out that none can be kept indefinitely without de-emulsification setting in. For practical purposes it is sufficient to study their behaviour after comparatively short periods of time following their manufacture. If they will remain stable for one hour it is sufficient for practical purposes and experience shows that if any decided de-emulsification is going to set in it does so in the first hour. In the laboratory, therefore, after the various emulsions had been tested for wetting power they were poured, after thorough agitation, into burettes and allowed to stand for one hour.

It was found that all those formulae containing only $\frac{1}{2}$ % of soap in tap water were unstable and therefore dangerous to use. This agrees with what might be expected from the plotted results of wetting power, where the line indicating wetting by paraffin is always equal to or above the line indicating wetting by aqueous solution. Similar emulsions made with distilled water were stable even in cases (paraffin 10 %—25 %) where, in the plotted results, the paraffin wetting line is above the water wetting line. These emulsions therefore, though not certain to scorch, cannot necessarily be classed as safe. With 1 % soap and tap water the emulsions were still slightly unstable, as might be expected from the plotted results where the paraffin wetting line is nearly always above the water wetting line. These emulsions are therefore dangerous except in those cases where the paraffin content is low. Most plants will stand a very small quantity of free paraffin if well distributed, since, under conditions of spraying, much of it is evaporated before it can produce scorching effects. When however much is present scorching is bound to occur. The other emulsions tried, namely 1, $1\frac{1}{2}$ and 2 % with distilled water and $1\frac{1}{2}$ and 2 % with tap water, gave stable emulsions. It will be seen that in the case of $1\frac{1}{2}$ % soap and tap water at 20 and 25 % the paraffin wetting line was above the water wetting line, indicating that the emulsion was unstable. Actually however when tested for an hour in a burette no appreciable de-emulsified paraffin appeared. This should not be taken to indicate that it is a safe emulsion to use. It contains a high content of paraffin which is only just held in emulsion by the soap. In other words the soap pellicule that separates the paraffin globules is nearly as thin as it can be without actually parting asunder. So long as the liquid is at rest it is stable, but when forcibly beaten against a solid object as in spraying,

the soap pellicules break and liberate a certain amount of free paraffin. Such an emulsion is therefore unsafe to use. It is thus possible to divide emulsions into three classes:

1. Dangerous. 2. Unsafe. 3. Safe.

The first includes those which, like the $\frac{1}{2}$ % soap with tap water, de-emulsify on standing. The second, those like $1\frac{1}{2}$ % soap with tap water and 20 or 25 % paraffin, which de-emulsify on being sprayed. The third, those like the 2 % soap emulsions, in which no de-emulsification occurs at all.

Wetting Power of Paraffin Emulsion compared with other Auxiliary Wetting Agents.

In the previous portion of this paper the wetting power of paraffin emulsions on a certain artificial surface, American cloth, has been considered. Table III gives the results of the wetting power of some of these emulsions and of other auxiliary wetting agents on certain natural surfaces.

TABLE III. *Wetting Power of Various Fluids.*

Wetting Substance	Gooseberry Leaf	Sea-kale Leaf	American Gooseberry Mildew
Water	Nearly complete	None	None
Gelatine 1 in 1000	Complete	Very slight	,,
Gelatine 1 in 10,000	,,	,,	,,
Casein 1 in 1000	,,	,,	,,
Casein 1 in 10,000	,,	,,	,,
Soap $\frac{1}{2}$ %	,,	Slight	,,
Soap 1 %	,,	Nearly complete	Very slight
Soap 2 %	,,	,,	,,
Emulsion Soap 1 %, Paraffin 1 %	,,	,,	Moderate
Emulsion Soap $1\frac{1}{2}$ %, Paraffin $1\frac{1}{2}$ %	,,	Complete	Nearly complete
Emulsion Soap 2 %, Paraffin 2 %	,,	,,	Complete
Emulsion Soap 1 %, Paraffin 5 %	,,	Wets by paraffin but not by aqueous solution	Nearly complete
Emulsion Soap 2 %, Paraffin 1 %	,,	Complete	Moderate

These surfaces were chosen to represent types that could be wetted with ease, with moderate difficulty and with great difficulty. They are a gooseberry leaf, a sea-kale leaf, and the white felted stage of American gooseberry mildew. The gooseberry leaf is smooth without being waxy and is easily wetted, the leaf of sea-kale is slightly waxy and is only wetted with moderate difficulty, while the surface of American gooseberry mildew offers great difficulty.

Of the other wetting agents soap, gelatine and casein were tested. Both the latter have recently been recommended, the former in an acid or neutral medium, the latter in an alkaline. For both one part in a thousand is relatively strong while one part in ten thousand is relatively weak; they were therefore tested at those strengths. Even at the weaker strength both increased the wetting power but the increase was only slight. They caused complete wetting of the gooseberry leaf but only raised that of the sea-kale leaf from none to very slight while the effect on the mildew was nil. For waxy and hairy surfaces, therefore, they are useless though they are to be recommended where extra spreading power is desired on an easily wetted surface. With arsenate of lead and more especially with Bordeaux mixture, where a cheap substance is required to obtain even spreading, they are most efficient.

Soap and paraffin emulsions having a soap basis may be considered together as they can only be used in mixtures where soap gives no chemical reaction. Where soluble salts of bases other than potassium, sodium and ammonium are present insoluble stearates are formed which prevent the use of soap. Half a per cent. of soap proved itself slightly superior to both gelatine and casein while the higher percentages gave nearly complete wetting on sea-kale and a very slight wetting on the mildew. It is clear, however, that the use of soap is limited as, even at 2 % strength it proved itself inefficient when tried on the most difficult surface. The paraffin emulsions proved themselves much more potent.

The strengths tried were as follows:

Paraffin Emulsion	1, 1
,, ,,	1½, 1½
,, ,,	2, 2
,, ,,	1, 5
,, ,,	2, 1

In each case the figure first given is the percentage of soap and the

second that of paraffin. (Thus P.E. 2, 2 indicates Paraffin Emulsion, 2 % soap = 20 lbs. to 100 gallons of water and 2 % paraffin = 2 gallons to 100 gallons of water.)

P.E. 1, 1 gave superior results to 2 % of soap in that it gave better wetting of the mildew. P.E. $1\frac{1}{2}$, $1\frac{1}{2}$ was still better while P.E. 2, 2 gave perfect wetting. P.E. 1, 5, though it nearly completely wetted the mildew, was found to wet by means of paraffin instead of by aqueous solution and so, though a cheaper mixture than P.E. 2, 2 would have great danger of scorching foliage. P.E. 2, 1 stands intermediate in wetting power between 2 % soap and P.E. 2, 2 and shows the influence of introducing emulsified paraffin on the wetting properties of the liquid.

There is no object in introducing greater quantities of either paraffin or soap as P.E. 2, 2 gives perfect wetting. On the other hand the behaviour of P.E. 1, 5 and P.E. 2, 1 show that it is not possible to reduce the quantities of either without destroying the desirable qualities of the mixture. P.E. 2, 2 is, therefore, the cheapest mixture that can be used which at the same time has the highest wetting power. The value of this 2 % emulsion lies not so much in its own killing power as in the fact that it can act as a carrier, so to speak, for other fungicidal or insecticidal bodies which, used alone, would prove themselves insufficient to kill. Thus liver of sulphur, used alone, has no great controlling effect on American gooseberry mildew but, combined with paraffin emulsion, has given promising results in a commercial scale experiment undertaken by Prof. Barker and myself. In the direction of insect control it also shows promise. While dilute solutions of nicotine are without decided action on adult caterpillars or difficultly killed beetles such as *Byturus tomentosus*, the Raspberry Beetle, it has been found possible, at any rate on the small scale, to kill these by uniting the same nicotine solution with 2 % paraffin emulsion.

EMPUSA MUSCAE versus *MUSCA DOMESTICA* L.

By H. T. GÜSSOW,

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(With Plate XXI.)

The biological control of insect pests has received considerable attention for some years past, and success has without doubt accompanied research work along these lines. The common house fly (*Musca domestica*, L.) is more than ever now, because of additional proof furnished by more recent investigations into their responsibility as carriers of wound infections, recognised as an important source of danger to public health.

The most destructive disease, to which the house fly is subject, is that caused by the fungus *Empusa Muscae*. The question whether this fungus may be used in the control of this pest is of decided interest, and has received more or less careful attention from writers, as well as from investigators.

It has already been pointed out in an earlier paper[1] that it is an easy matter experimentally to spread the disease among flies in captivity. That is to say material from flies recently dead from the disease produced infection in others brought into contact with the spores of *Empusa*. Yet, fatal as the disease is among flies, it was for long a matter of conjecture whether it really constituted an important factor in the death of flies. No doubt the very fact of its being fatal, coupled with the number of dead flies to be seen every year towards autumn, helped to attach an importance to this disease, which a close study failed to confirm. Close observations made in places, where flies are present by thousands throughout the season, indicate that perhaps one in a thousand flies is killed naturally by the disease,—in some instances even a smaller proportion. The determination of the mortality among flies from this disease is comparatively simple, since, as a rule, the fly killed by *Empusa* is and remains attached to

[1] Güssow, H. T., "*Empusa Muscae* and the Extermination of the house fly." *Rep. Loc. Govt. Board, on Publ. Health and Medical Subjects.* New Series, No. 85, p. 10.

the substratum where it died. In the localities referred to, many thousands of flies frequented the places daily, but only from 1 to 4 at the most of newly deceased flies' bodies were observed on any one day during a period of 22 days. Hence in nature this fungus does not appear to account for the general disappearance of flies in winter.

Though I am quite satisfied that one cannot speak of this disease as the cause of an epidemic among flies, and though, endemically, it may be present every season, the fact that it is so fatal to individual flies made it desirable to experiment with the fungus with a view to spreading the disease more widely and rapidly. This might be done were it possible to grow the fungus conveniently in artificial culture, and use it in the manner of, for instance, D'Herelle's *Coccobacillus acridiorum*, pathogenic to grasshoppers.

Methods of securing artificial cultures have been tried by a number of observers, and because of some recent publications on the subject, which, perhaps, have not come to the attention of scientific workers, it will not be out of place to discuss the opinion of these writers here. In the last note which I prepared (*loc. cit.*), I briefly dealt with the investigations of Mr Edgar Hesse, on the subject of culture of *Empusa*.

The seventh report of the same Board (No. 102, 1914) contains further contributions to the subject under discussion. In the letter of transmittal Dr Newsholme, the medical officer of the Board, briefly reviews the original contributions in the report. We now take exception to the statement that the *Empusa* "is known to be the cause of much of the mortality among the house flies during the autumn months," because of careful observations on the rate of mortality. Notwithstanding my friendly criticism of Mr Hesse's 1912 work in the sixth report, his work receives much support from Dr Bernstein who was appointed by the Board "to supervise and control Mr Hesse's investigations." It must be pointed out here that Mr Hesse claimed and "proved" (!) "the possibility of obtaining from spores of *E. Muscae* derived from flies which had died of the disease, a culture which, under suitable conditions as to moisture and food material, gave a luxuriant growth in a few days, followed by the production of spores. These spores when mixed with syrup and fed to normal flies, induced in them a rapidly fatal disease, which, as judged by the naked eye and by microscopical examination, was indistinguishable from the disease found in the flies attacked by *Empusa Muscae* in the ordinary course." Mr Hesse, be it noted, refers to luxuriant growth and production of spores, but does not here state what growth or spores.

When, through the courtesy of Dr. C. Gordon Hewitt, some original material of Mr Hesse's culture was placed at my disposal, I could, on examination, find nothing else but *Mucor racemosus*, which certainly produced after a few days a luxuriant growth of the same fungus: we also observed "spores," but not *Empusa* spores. Since that time experiments have been made with spores of this universal mould fungus on living flies, but, notwithstanding an ample supply quite sufficient to fatten them, they died of starvation; but under no circumstances was death due to *Mucor racemosus*, since sound flies, kept under identical conditions minus the fungus, died about the same time from the effects of confinement. Mr Hesse solved the difficulty apparent in this instance by concluding that this fungus was polymorphic, and "that *Empusa* was merely a parasitic form of the *Mucor*." Mr Hesse supplements this statement by the following paragraph:—

"The idea that the *Empusa Muscae* might be polymorphic now presented itself, and also that this might be the reason that its cultivation had not apparently been successful hitherto. I realised that one should not expect to produce a purely parasitic growth on a dead medium (*sic!*), but that, as in the case of other fungi when artificially cultivated, a morphological change might be expected; and came to the conclusion that *Mucor racemosus*, although a saprophyte, might, when the spore is ingested by the fly, assume a parasitic existence and produce the growth, in the tissues of the fly, known as *Empusa Muscae*, whose spores, when cultivated on artificial media, revert to the saprophytic stage."

It is regrettable to notice the author's attempt to interpret an error of his by proposing a somewhat fantastic theory. Surely before such explanation is necessary, he should be sure that what he recorded as occurring did actually occur, viz.: the growth of *Mucor* from germinating *Empusa* spores. It is so clear that it hardly needs stating that he has confused an adulteration of his "cultures" with what he thought he had produced. The use of an egg yolk medium "in thin layers in a Petri dish, sterilised by steam at 100° C. for one hour on three days in succession," clearly indicates the degree of importance attaching to the author's further results, whatever they may be.

He proceeds then by inserting bodies of flies which had died of *Empusa* during the previous autumn. Control dishes were also prepared. In five days sporangia had formed on the dishes containing the egg medium, whilst the control dishes remained quite sterile. The author, unfortunately, does not indicate whether he placed on his

control dishes dead flies, not infected with *Empusa*, but at any rate collected at a similar date (the previous autumn), which precaution one would naturally expect in controlling the results of the first experiment. It appears that he placed no flies whatsoever on his control dishes, which naturally remained sterile. Had he done so, we are prepared to predict the identical formation of sporangia observed in his experiments with *Empusa* killed flies.

Next he made transfers from his culture, sporangia forming in the secondary culture. "Meanwhile," he states, "the fungus of his secondary culture, which in every respect was identical with *Mucor racemosus*, was transferred to a syrup made of sterilised sugar and water, and put aside for experiments." How long he does not say. Nor whether the spores had time to germinate in the sugar solution in the "meantime."

Now comes the most surprising effect! In his experiments Mr Hesse uses "flies all bred from insects in confinement"; in June "the chrysalides of the greater and lesser flies commenced hatching out" (? *Musca domestica* L. the common house fly, and *Fannia canicularis*, L. the lesser house fly). In his first experiment all negative results were secured, owing apparently to absence of plum jam. Notwithstanding an earlier statement by the author that many insects became entangled in the sticky mess of plum jam, in the second experiment this was used together with, though not specifically so stated, a filter paper saturated, as in the first experiment, with a syrup in which had been incorporated spores (we take it *Mucor* spores). By the fourteenth day the first deaths were noticed; by the twenty-first day all flies were dead. "All presented typical outward (*sic*) appearances of infection with *Empusa Muscae*." No control was made in this case, he states.

In experiment three,—a repetition of No. 2,—the first deaths occurred in four days, and by the eleventh day all were dead. Cause of death is not stated.

In the fourth experiment there was a control with flies not fed with "spores," and some fed with spores from the fourth generation of *Mucor*. "The results were the same" (as which?). In the control "no deaths occurred and the flies appeared to be normal four weeks later." This is most remarkable; and, if Mr Hesse had not reported this as an actual observation, I should feel inclined to challenge the accuracy of these observations.

Cultures were then made by this indefatigable worker from "flies which had died in the cages with the naked eye appearance of infection

with *Empusa Muscae*." These resulted in a prolific growth of *Mucor racemosus*. Why, I wonder, the cautious expressions of "outward" and "naked eye" appearances? One must instinctively ask:—Was *Empusa Muscae* present, or did it only appear to be present? A statement of this most important fact is carefully avoided. Why?

Dr Bernstein thus adds his opinion: "There can be no doubt than that Mr Hesse has succeeded in producing a growth, ingestion of the spores of which results in the death of all the flies from *Empusa Muscae*." One cannot but feel greatly surprised at such a statement from the nature of the research work quoted.

After consultation with Dr Copeman and Mr Ramsbottom (of the Nat. History Museum) the investigators, Dr Bernstein states, agreed on this fact. Here I should wish to ask:—Did they agree on the fact that the flies dead from *Empusa Muscae* were the flies used, or did they merely agree that all the flies, presumably submitted for examination, were dead from *Empusa Muscae*?

Dr Bernstein now steps in the breach and tries a number of experiments. Cage 1 was not interfered with; Cage 2 received a paper saturated with sterilised syrup of cane sugar; Cage 3 received in addition a paper saturated with syrup containing the spores of *Mucor hiemalis* supplied by Mr Ramsbottom. Cage 4 contained a paper saturated with syrup containing spores of *Mucor racemosus*, "which had been cultivated on egg yolk from *Empusa Muscae* (*sic*). The cages were kept in a warm room (in whose charge, or under whose observation, we would gladly have seen stated). Abnormal changes only occurred in Cage 4. In fourteen days 75 % of the flies were dead. Mr Ramsbottom, examining one of the flies from this cage, expresses his opinion that the manifestations were identical with those of *Empusa Muscae*. No fly died in the other cages from *Empusa*.

The general conclusions are stated as follows:—"It would seem then that there could be no doubt that the deaths of the flies in Cage 4 were due to a fungus indistinguishable from *Mucor racemosus*, but which can be readily cultivated in great quantities from the bodies of flies dead of *Empusa Muscae*." This is more correct; we note "from the bodies of flies dead of *Empusa*"; what rôle did the *Empusa* play therein?

Empusa Muscae spores under no circumstances have produced in the cultures made by myself and many other workers anything else but what belonged exclusively to that fungus; certainly never anything like *Mucor racemosus*. *Mucor racemosus*, or *Mucor* resembling what

may now be somewhat loosely called *Mucor racemosus*, was readily secured from the bodies of any dead fly. We cannot, of course, doubt the correctness of the recorded observations; but we are of the opinion that if a *Mucor* proved fatal to flies in the manner described in these experiments, it must have been one of the pathogenic types or a different species altogether. We expect close diagnostic studies of the pathogenic organism would soon establish the identity. But we do absolutely question that *Mucor* developes from an uncontaminated *Empusa* spore. We are glad to note that Mr Ramsbottom has now become interested in clearing up this somewhat involved research work; and we quite agree with him that -if Mr Hesse's assumption is correct that *Empusa* is a polymorph --a fundamental biological principle would be absolutely overthrown.

The critic, they say, but assumes the rôle when he has failed in producing good or marketable merchandise in his own line. That I have assumed the critic's rôle, I may not deny: in what follows, I would play a constructive part, with what success I modestly leave in turn to my critics to say.

I wish, therefore, in concluding to record some of my cultural experiences and other observations on *Empusa Muscae*, which I have not hitherto published. This may assist in simplifying the apparent difficulties which Mr Hesse had in securing an uncontaminated growth from *Empusa* spores.

In my last paper (*loc. cit.*) I described briefly a method of successful auto-infection of flies coming into contact with flies recently dead from *Empusa*. By this means one has under one's own control the securing of fresh material of the fungus for a considerable period. By placing a fly showing the fresh belts of fungus growth on a pillar made of plasticine, this latter can be bent and twisted under the microscope into any position. When carefully adjusted one can observe the discharge of spores very readily. These fly in every direction and for distances of about seven centimetres.

Hence this fact was used for obtaining spore cultures quite easily, and uncontaminated. A series of clear coverglasses was placed at the bottom of a sterile jar, which was closed with an ordinary cork. A fly which was observed to be surely "infected"—experience having shown the incipient symptoms of infection, viz. sluggishness, increase in volume and lightening in colour of abdomen, and a peculiar reddish colour, a dirty brick red, of the "eyes"- was pinned on to the cork from below, so that, when the stopper was replaced, the fly was securely held above

the coverglasses underneath. Spores were freely shed and collected absolutely pure.

The usual methods used in spore cultures were then employed, but the results were not satisfactory. I then used for a medium various animal fats—a droplet of butter, of dripping, of lard, etc. These were exposed to the spore bombardment, but no more appreciable results were obtained. Moreover, these substances were found to be very unsuitable for microscopic use owing to their annoying refractive indices and their opacity. How much more unsatisfactory must the egg yolk medium—stone hard, no doubt, from the method of treatment given by Mr Hesse—have proved itself. I also tried sterile asparagus tips—mainly because of the known lecithin contents of this plant—but no satisfactory results were achieved on it either.

I then obtained interesting results from the use of a mineral fat, i.e. common vaseline, as used for ringing slides in hanging drops. This medium was sterile to begin with and made beautiful clear hanging drops or surfaces. A small quantity was placed with a scalpel on a clean coverglass and gently heated until evenly spread like a film over the coverglass. When exposed to spore-shedding, the number of spores desired thereon was easily adjusted by the removal of coverglasses after one, two, three or more minutes' exposure. The coverglasses were then inverted on hollow ground slides and ready for examination.

To obviate any misunderstanding, here let me remark parenthetically that, in the description following, I am not detailing the result of any one particular experiment, but am summarising the nett results of some hundreds of observations.

Germination began very shortly in every instance. The plasmatic mass which surrounds the spore when shot from the conidiophore appeared to remain in a liquid or semi-liquid form; it never showed the wrinkled appearance generally observed, and so often figured. The first symptoms of active life appeared about one hour after the spores had been shot on the medium. Never at any time did the original spore produce a germinal tube; at first there appeared something very much like a germinal tube in the shape of a small "exdentation" of the cell wall of the mother spore. This gradually elongated, but assumed, from ten to fifteen minutes later, a flask or clubshaped appearance. About an hour later it was recognised with certainty as a secondary spore, the nucleus of the first spore having taken up its position in the now forming secondary spore. As this spore grew, it, together with the mother spore, presented a dumb-bell shape. Where joined

to the mother spore the "handle of the dumb-bell" elongated somewhat, and the secondary spore also appeared surrounded by a plasmatic substance—just as did the mother spore—but in very much smaller quantity.

Immediately after the secondary spore was formed, it actually did germinate. It should have been said that in the mother cell the "exdentation" occurred on almost any position of the spherical body. But with the secondary spore, germination, as opposed to the "exdentation" of the mother cell, took place at the end towards the mother cell. Indeed what appeared at first as a rather long "handle to the dumb-bell" was later recognised as a mycelial thread, or, more correctly, tube growing into the cavity of the old spore. Naturally in many cases the secondary spore often separated from the mother spore.

The germ tube was seen to branch, and the contents of the spore to be slowly used up, while the mycelium grew in size. This produced sparse, short, stout branches at no special points. Later on a second germ tube grew from the secondary spore body; on rare occasions there were three in all. The growth made slow progress thereafter, becoming detached from its spore shell, and assuming shapes and sizes similar to the mycelial portion observed in the fat body of the fly. We have not been able to see a true tertiary spore in *Empusa Muscae* after germination took place, although peculiar club or flask shapes occasionally appeared which resembled spores; but they could not, on examination, be determined as such.

The growth remained pure all the time and made progress for 28 days on this medium. Then signs of disintegration appeared, and the mycelium became vacuoled more and more, less sharp in outline, and later collapsed. This is, so far as I know, the longest time a growth has been maintained outside a fly; but we cannot regard it as a successful culture yet. Every effort to continue growth failed, no doubt because it normally takes place in nature in the living fly body.

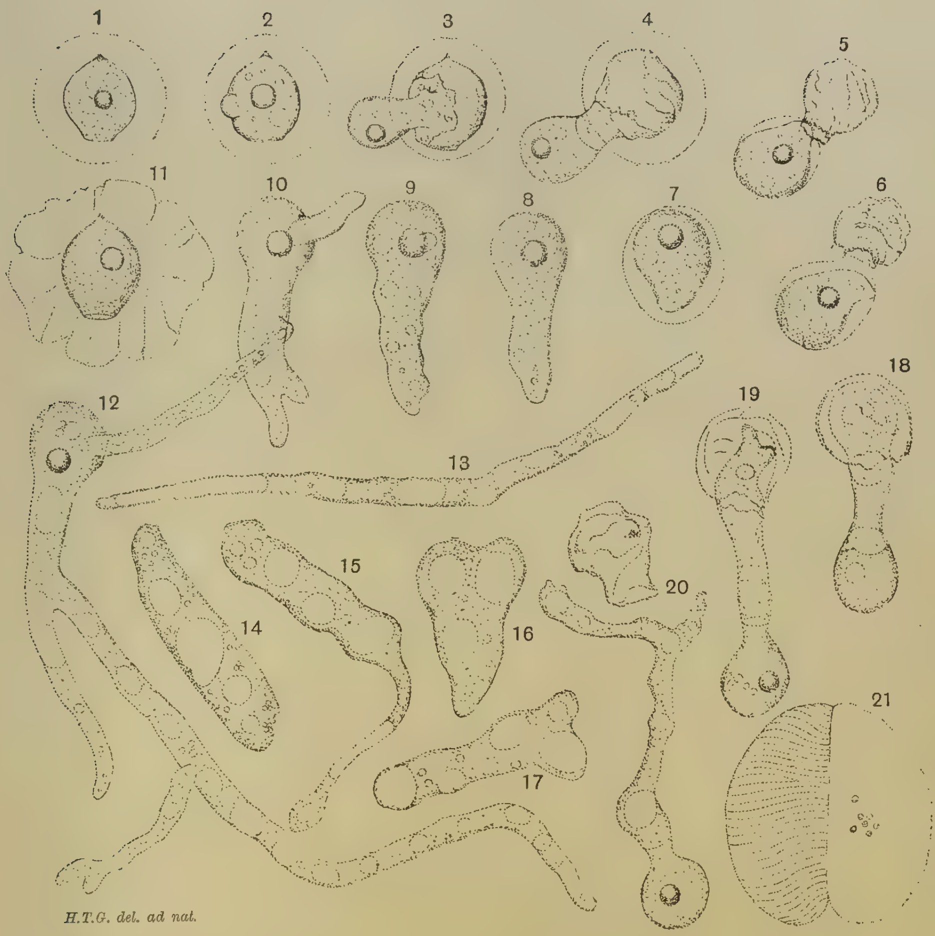
These observations clearly show that Mr Hesse's *Mucor racemosus* was nothing else but an impurity, and polymorphism does not occur in this fungus, as represented at one time by *Mucor* and another by *Empusa*.

Every other experiment to continue the development of these spores has failed so far. The absence of nutrients accounts for this; for the various ingredients tried to furnish material for continued growth did not suit the fungus. The main points, from a scientific

point of view, viz. the hibernation, the question of resting spores, so common in other species of this genus, and of artificial culture,—still remain unsolved. But, while it is realised as possible that the solution of these problems may have other than a scientific value, it appears to me from my observation that we must look, for the control of the fly problem, to other biological organisms—or remove systematically, by all necessary precautions, the insanitary conditions favourable to the breeding of this annoying and dangerous pest.

EXPLANATION OF PLATE XXI.

Fig. 1. Empusa spore shot on vaseline at 9.15 a.m.
Fig. 2. Same spore at 11.15 a.m.
Fig. 3. „ „ 12.10 p.m.
Fig. 4. „ „ 12.25 p.m.
Figs. 5, 6. Same spore, a little later.
Fig. 7. Same spore at 4.40 p.m.
Fig. 8. „ „ 5.20 p.m.
Fig. 9. „ „ 5.30 p.m.
Fig. 10. „ „ 6.5 p.m.
Fig. 11. Spore shot on glass slide, mass of protoplasm drying up.
Fig. 12. Same spore as before after 24 hours' germination.
Figs. 13–17. "Involution forms."
Figs. 18–20. Spore germinating into mother spore, and becoming separated from same.
Fig. 21. Proboscis of *Musca domestica* and spore of *Empusa Muscae*, relative sizes.



H.T.G. del. ad nat.

A BLOSSOM WILT AND CANKER OF APPLE TREES.

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(With Plates XXII—XXIV.)

CONTENTS.

I. Introduction.

During recent years many varieties of apples have been seriously attacked by a disease which causes the wilting and death of the blossom, frequently kills the twigs, and may produce cankers on the branches. From information received at Wye College from various localities in

Kent and Sussex it is evident that the disease is increasing in intensity year by year; in some orchards and plantations it has now assumed epidemic proportions and is causing considerable loss to the fruit farmers.

The varieties which have suffered most are Lord Derby, Cox's Orange Pippin and James Grieve. A Sussex fruit grower[1] who supplied the writer with specimens gave the following list of varieties affected with the disease in one of his orchards in 1915: Duchess of Oldenburg, Worcester Pearmain, Allington Pippin, Cox's Orange Pippin (this variety very badly attacked), Early Julyan, James Grieve, Lane's Prince Albert, Lord Grosvenor, Prince Bismarck, Chelmsford Wonder, Newton Wonder, Domino and Lord Derby. In the same orchard the varieties which the owner found to be free, or practically so, from the disease were Charles Ross, Gladstone, Beauty of Bath, Lady Sudeley, Blenheim Orange, Royal Jubilee, Bramley's Seedling, Warner's King and Queen. Whether any of the varieties included in the latter list are immune is at present an open question but some of them are known to be susceptible, for Beauty of Bath, Warner's King, and Bramley's Seedling have been found elsewhere with the disease, as have also Duchess' Favourite, Ecklinville Seedling, Fearn's Pippin, Dartmouth Crab and Rival. The same grower writing in May 1915 said, the disease "is more extensive among apples than I have ever seen it before. I reckon that in some varieties one-fourth of the trusses of fruit have been ruined by it"; with reference to a similar outbreak in 1916 he wrote, "The specimens enclosed are from Fearn's Pippin which is very badly damaged. One fairly large tree blossomed all over profusely and I am sure that nineteen out of twenty of the trusses of fruit blossom have gone off like those enclosed," and in another letter he averred that "Three-fourths of my anticipated crops of Cox and Domino, and half that of some other varieties have been destroyed by the disease."

Inspection of affected orchards and plantations in Kent during the past season (1916) has shown that the experience of the Sussex grower is by no means unique, for trees with from 50 % to 75 % of the flowering spurs killed by the disease were not uncommon. On one farm visited by the writer there were thirty large standard trees, about twenty years old, of the Lord Derby variety which had produced about 300 bushels of fruit in 1914 when a little of the disease was noticed; in 1915 the disease was worse, while last season (1916) the crop was practically nil and hundreds of dead spurs recently killed were to be seen on each

[1] I am indebted to Mr Wm E. Bear, Hailsham, for the information supplied.

tree. The affected trees were in two rows; along one side of them was a row of the variety Warner's King bearing a few diseased trusses while along the other side the trees (Newton Wonder) were apparently quite free from the disease. This would appear to indicate that the Lord Derby variety is particularly susceptible to the disease and this conclusion is supported by the fact that in one large orchard where there were some hundreds of trees of this variety together with several other varieties it was possible to detect the Lord Derby trees even in winter by reason of the large number of dead spurs and twigs which they bore. In the Weald of Kent where this variety is extensively cultivated so much injury has been caused by the disease that it has been necessary in some cases to "top-graft" the trees with a more resistant variety.

Not only are well-established trees attacked, but quite young trees too are susceptible to the disease. In one case, observed in the fruit plantation at Wye College, a young cordon apple tree (of the variety Rival) was attacked through a fruiting spur situated near the middle of the stem during the first season after it was planted out; a canker developed round the base of the affected spur and killed the upper half of the tree.

The first symptoms of the disease are noticeable about a fortnight after the tree comes into flower; it will then be observed that some "fruiting spurs" of the trees affected not only fail to set fruit but the flowers and leaves round the base of the inflorescence show evidence of wilting, and, within a few days become dry and withered. Where such a truss is borne on a short spur there will be found about the middle of June a depressed, often cracked, canker-like area on the branch, around the base of the spur. In some instances the canker within a few weeks girdles the branch and so causes the death of that portion distal to the canker.

Usually no external sign of any parasitic organism is to be found on these newly killed spurs at this stage. During periods of wet weather however the dead flowers and pedicels may produce pustules bearing chains of elliptical to citriform conidia which are readily identified as of the *Monilia* type. Again if sections are made through the base of a dead truss, showing externally no trace of the fungus, and examined microscopically, hyphae are invariably to be found. When particles of these sections are placed on nutrient culture media the fungus continues to develop and may be induced to produce conidia when growing in pure culture; under these conditions too the organism is recognised as a *Monilia*.

If the dead spurs are allowed to remain on the trees until the following season the fungus appears at the surface of the spurs and over the cankered areas during the winter and spring in the form of rounded *Monilia* pustules which burst through the bark and produce numerous chains of conidia (Fig. 1).

The constant association of the *Monilia* with the Blossom Wilt and the appearance of that fungus on the diseased spurs and cankers obviously suggest that the organism is responsible for the damage done. The *Monilia* that is generally assumed by plant pathologists, in this country and abroad, to attack the apple is *Monilia fructigena* Pers. (= *Sclerotinia fructigena* Schröter). In its conidial form this is the fungus that so readily attacks the ripening apple during the late summer and often causes them to become "mummified." On such apples the fungus is usually to be seen in the form of *yellow* pustules which appear more or less in concentric circles over the diseased fruit. The form which I have hitherto always found to be associated with the Blossom Wilt was found to be quite distinct from the typical *Monilia fructigena* found on the fruit in summer and autumn, in the colour and size of its pustules, in the dimensions of the conidia and in its habit and growth on artificially prepared culture media. The pustules of the Blossom Wilt fungus are in general smaller than in *M. fructigena*, are grey rather than yellow in colour and the conidia they bear are smaller. This grey *Monilia* of the apple conforms more nearly to descriptions of *Monilia cinerea* Bon. (= *Sclerotinia cinerea* Schröter) which, according to those continental mycologists who recognise this as a species distinct from *M. fructigena*, is the form responsible for the majority of cases of Brown Rot in the "stone-fruit" trees (i.e. plum, cherry, damson etc.).

II. Historical.

In 1888 Sorauer (21) pointed out that not only was *Monilia* destructive to the apples themselves but that it could invade the woody tissue of the twigs which were in consequence killed towards their tips, and since that year frequent references to this "Zweigdürre" have appeared in continental phytopathological literature. This condition on apple trees is generally attributed to *M. fructigena*, while a similar disease on cherry trees is said to be caused by *M. cinerea* by those who recognise the two as distinct species.

Frank and Krüger (10) in 1899 make reference to an outbreak of Brown Rot on apple trees in the neighbourhood of Berlin; on the

affected trees fruiting spurs were killed soon after the flowers opened, the flowers and leaves became withered and the fungus penetrated into the bark of the branches.

One of the most valuable contributions to our knowledge of the Brown Rot *Monilias* is a paper by Woronin (23) published in 1899; in it he not only supplied evidence in favour of his contention that *Sclerotinia fructigena* and *S. cinerea* were distinct species and could be distinguished even in the *Monilia* (or conidial) condition, but also used the two forms in his inoculation experiments using, apparently, pure strains[1] of these fungi. With regard to his results when the flowers of the apple were inoculated, he found that the conidia of *S. cinerea* germinated on the stigmas and attacked the styles slightly but the germ-tubes were unable to penetrate further into the flower. The germ tubes of the conidia of *S. fructigena* on the other hand grew out into all parts of the flower and then into the pedicels and leaves, the latter gradually becoming withered. From these results he states emphatically, "Meine Experimente beweisen ausserdem, dass die Laubdürre bei den Aepfeln nur durch *Sclerotinia fructigena* verursacht werden kann."

In 1900 Aderhold (1) records having received specimens of apple twigs which, from their appearance, he believed had been destroyed by "fire-blight," the bacterial disease which ravages pear and apple trees in America. The affected shoots contained a white mycelium which when cultivated in hanging drops produced a *Penicillium*-like fructification[2] which he considered was the conidial form of a *Mollisia*. Later however (2) having found that shoots killed during the previous year bore *Monilia* pustules he attributed their death to the action of *M. cinerea* and concluded that the form previously observed was of secondary origin.

Müller-Thurgau (15) in the same year describes a similar disease of apples and pear trees but refers the causal organism to *M. fructigena*.

Aderhold and Ruhland (4) in 1905 confirmed the work of Woronin in that they found that inoculation of apple flowers with *Sclerotinia cinerea* led to a weak infection while *Sclerotinia fructigena* readily produced the death of the blossoms.

Eriksson (8) in 1913 figured and described a canker-like disease of

[1] Woronin is not quite clear on this point; on p. 18 he writes of "zahlreiche, reine, streng controlirte und möglichst variirte Impfversuche mit diesen beiden Pilzen," but whether his "reine Impfversuche" were made from cultures growing on previously sterilised media is not stated.

[2] From the description and figures it would appear that these *Penicillium*-like fructifications consisted of "sporidia" or "microconidia" of the *Monilia*, as the production of these bodies is readily induced in artificially prepared cultures of the Brown Rot fungi.

apple trees which, from the description, appears to be identical with that observed in this country. He found that the *Monilia* pustules which developed on the affected parts were grey in colour but states that he makes no distinction between *Monilia cinerea* and *M. fructigena*, and assumes that the form which appears on the fruit in autumn is a stage in the annual cycle of that form which kills the twigs and blossoms.

Broz (6) in 1913 describes the occurrence of the "Zweigdürre" at Vienna and states that late frosts favour the outbreak of the disease.

Brown Rot is also known in America where it is particularly destructive to peach trees, killing blossom, fruit and branches. The American form, though it has been generally[1] named *Sclerotinia fructigena*, is found by the more recent investigations of Matheny (14), Jehle (12), Conel (7), and Bartram (5) to conform more nearly to the descriptions given of *S. cinerea*, and is referred by them to that species.

In our own country it has been customary to refer all cases of Brown Rot of fruit trees to *Monilia fructigena*, and although the *Monilias* are probably responsible for greater losses to the English fruit growers than any other genus of fungi (since in one form or another it attacks the blossom, young fruit, ripening fruit, fruit in the store, twigs and branches), no attempt appears to have been made until quite recently to determine whether the conclusions arrived at by Continental workers hold good for Britain.

In 1903 Mr G. Massee observes (13) that *Monilia fructigena* "is most frequently seen on apples and although best known to the casual observer on the fruit occurs also on the young shoots, leaves and even the flowers." That *Monilia* is capable not only of killing the flowering spurs but may also produce large cankers on the main limbs of apple trees was recorded by Mr Salmon (16) in 1910. Since that year many specimens of Blossom Wilt and Brown Rot canker have been sent to Wye College, and it was evident that the disease was becoming a serious menace to the cultivation of some varieties of apples in certain districts, particularly in Kent.

Mr Salmon has, on several occasions[2], pointed out the serious nature of this form of Brown Rot; since preliminary examination showed that the results of investigations made abroad were not wholly in accordance with observations made on the disease as affecting apple trees in this country it was evident that it was a subject demanding further research, and as Mr Salmon himself, from pressure of other duties, was unable to continue the work, it was entrusted to the present writer.

[1] *Vide* Duggar's *Fungous Diseases of Plants*, footnote on page 187.

[2] See Bibliography on p. 203.

The investigation is still in progress but it was thought desirable that the facts already ascertained should be published in order that steps might be taken to check the further spread of the disease.

III. The Disease as observed on naturally infected trees.

(a) *Observations made in* 1915.

An opportunity for studying the disease under conditions favourable for close examination occurred in 1915 when a row of apple trees in the fruit plantation at Wye College was found to be attacked by the Blossom Wilt. The trees, forty-eight in number, were of the varieties Warner's King and Duchess' Favourite planted alternately and were "closely spur-pruned" bush trees about eight feet high. Detailed observation commenced early in June 1915 and the following facts were noticed:

Not one of the trees was entirely free from Blossom Wilt though two of them had but one dead truss each, while the tree which had suffered most had 132 wilted trusses of blossom or about one-third of the total number present. The affected trusses (inflorescences) were recognised by their brown and withered drooping flowers and leaves; they were often greyish since the edges of the withered leaves showed a tendency to curl inward thus exposing the hairy under surface of the leaves. That some disintegration of the tissues had occurred was evidenced by taking a spur between the thumb and finger, immediately below the insertion of the inflorescence, when it was found that, when pressure was applied, the diseased spurs were more easily compressed than was the case with healthy spurs, and wilted trusses were easily broken off at the lower limit of the year's growth. The short axis of the inflorescence was at this time quite dead and when cut across was brown throughout; microscopic examination showed the presence of mycelium in the cortex, xylem and pith, the last sometimes being almost replaced by interwoven hyphae. The discoloration of the tissues extended to the older parts of the spur particularly in the cortical region and frequently reached the branch bearing the spur.

Some of these trusses, shortly after wilting, produced *Monilia* pustules on the dead flowers during a period of wet weather but in the majority the mycelium within the tissues apparently remained sterile throughout the summer. When however some of the latter were broken off from their spurs (i.e. at the lower limit of that year's growth) and placed on damp filter paper in a large petri dish, pustules of conidia readily appeared on the exposed broken end and on the withered flowers;

in some cases well-developed chains of conidia were produced within twenty-four hours after placing the dead trusses in the moist chamber. Pustular outgrowths were observed on some of the recently produced (from infection in 1915) cankers in August but they were barren and no conidia were found on them before December.

Old dead spurs bearing conspicuous pulverulent pustules of conidia during the summer months were present on some of the trees in the row, and in those cases where cankers had been caused these too had the conidial tufts scattered over the cankered surface, particularly towards the periphery. These in all probability were the result of infection during the previous flowering season of 1914. That these dead spurs and cankers were the source of infection resulting in the blossom wilt of 1915 and that the fungus on them was the cause of the injury was suggested by the fact that the newly killed trusses were most numerous on those trees bearing the greatest number of old spurs and cankers and were most frequently met with in their vicinity. Trusses within a short distance below such spurs and cankers were particularly liable to infection; thus on one branch of a Duchess' Favourite tree a portion one foot in length bore nine dead trusses, three above and six below a dead spur with its accompanying canker on which were numerous conidial fructifications of the *Monilia*—three of the dead trusses were on the spurs immediately below the canker. (This canker is shown in Fig. 2.)

The relation between the number of dead spurs and the number of wilted trusses per tree showed, although the disease may spread from affected trees to others in the neighbourhood, it may be stated generally, particularly if the trees are well separated from each other, that the wilted trusses are infected from spurs or cankers on the same tree.

The forty-eight trees in the row had in all seventy dead spurs (some with cankers) bearing the fungus; the number of dead trusses was 1319, and since there was no other source of infection in the immediate vicinity it must be assumed that the wilted trusses of these trees were infected from the seventy dead spurs; that is to say that in that year each old spur was responsible on the average for the death of nineteen trusses of blossom. A search in the neighbourhood of a group of closely aggregated dead trusses almost invariably revealed the presence of one or more dead spurs bearing the fungus.

Infection occurred through the flowers and not through the leaves as shown by the fact that primarily the flowering trusses only are attacked. On one of the trees (variety Warner's King) was a branch

with a single dead spur, near the upper end, bearing a number of powdery pustules; all the flowering spurs, fifteen in number, on that branch below the dead spur were killed, while all the barren spurs (i.e. bearing leaves only), eleven in number, were unaffected. Barren spurs and young vegetative shoots were not attacked directly but became wilted when borne on that portion of a branch distal to a canker which had encircled the branch, or when borne on a spur which also carried one or more infected trusses.

By the middle of June it was found that where diseased trusses were borne singly on short, simple (unbranched) spurs, 0·5 cm. to 1·0 cm. in length, growing from the younger portions (5–7 mm. in diameter) of the branches the fungus had traversed the tissues of the spurs and penetrated to the branch itself producing a canker completely encircling it and causing the wilting and death of those parts beyond the canker. On older portions (to about 1·5 cm. in diameter), bearing simple spurs to 2 cm. in length, the canker at the base of each affected spur had reached about half way round the branch.

A similar condition obtained in the case of spurs killed during the previous season, and it may be stated generally that when infection had occurred through a spur to 3 cm. in length a well-defined canker was produced extending upwards and downwards along the branch for several centimetres; the lateral extension of the canker was less than the longitudinal but on those portions of the branches two or three years old it was sufficient to completely girdle them. When the spurs were longer they were often killed to the level of their insertion on the branch, but on those 5 or 6 cm. in length the fungus as a rule had failed to reach the branch and the lower portions of such spurs were still alive.

The fungus may grow downwards from the infected truss along one side of the spur while the other side remains alive and unattacked. One spur had pustules over an area extending two-thirds round it but at the apex there were two young growing apples. On another the pustules had developed over the terminal portion and along the whole of one side, the other side being still alive for about half its length as shown by a living shoot near the middle; a section across this spur showed the tissues on the one side to be brown as far as the pith, while on the other they were still green.

A few old cankers were found from which the layers of dead tissue were in process of being removed by the formation beneath them of callus which was growing over and healing the cankered surface. These cankers at this stage showed no trace of the fungus but they bore such

a general resemblance to the *Monilia* cankers that it seemed probable that they had been caused by the fungus, whose further development had been arrested. The condition of such cankers together with observations made subsequently on younger cankers suggested that the fungus did not survive after it had produced its crop of conidia. In support of this it was found that the formation of callus had already commenced beneath the bark of cankers still bearing the pustules of the fungus (Fig. 8).

The observations made in June 1915 and recorded above suggested the following hypotheses:

(1) Infection arises from conidia falling upon the open flowers.

(2) The conidia are produced on spurs killed during the previous flowering season and on cankers arising round the base of the dead spurs.

(3) When the fungus has produced its crop of conidia on a spur or canker its further development ceases and in the case of cankers the injured surface becomes healed over by a growth of callus.

That the fungus was a true parasite and able to produce both the Blossom Wilt and Cankers was definitely proved in 1916 by means of inoculation experiments with pure cultures as is shown later in this paper. The other two points were established by attaching numbered labels to affected spurs and cankers, and recording observations at certain periods. After a few weeks this revealed another interesting feature relative to the cankers. It was found that not only did the older cankers (i.e. those produced in the previous year) show no further extension of the injury produced, but that the newly formed cankers also ceased to increase in size quite early in the season; many of them had attained to their maximum size by the middle of June while in others a slight increase in the cankered area could be detected towards the end of June or early in July, but in no case did a canker show any extension after July 12. It would appear therefore that the fungus penetrates no further into the tissues of the host after some six or eight weeks from the inception of the disease.

(*b*) *Observations made in* 1916.

The observations were continued in 1916, those spurs and cankers labelled in 1915 being examined from time to time while other cankers produced during May and June 1916 were also labelled; in all a record was kept of over 100 spurs and cankers and in no case was there any evidence that the fungus was able to make any further advance into

the tissues of the trees after the end of June of the year in which infection occurs. This was found to obtain not only for the two varieties Warner's King and Duchess' Favourite, but also for Lord Derby and James Grieve of which there were a few trees with the disease in the same plantation.

On the cankers produced in 1915 conidia were first found early in December of that year, and during January 1916 a considerable number of spurs and cankers showed pustules of conidia which proved to be viable and able to germinate at the temperature of the open air. On January 27 two hanging drops of distilled water containing conidia from separate spurs were set up and placed outside in the open air. Within 24 hours many of the conidia in both drops had germinated and had produced germ tubes up to 32μ in length.

It was found necessary during the winter to remove the Duchess' Favourite trees interplanted between the successive trees of Warner's King, so observations during the flowering season of 1916 were almost confined to the latter variety; two Duchess' Favourite trees were however retained and as the disease followed the same general course on these and on a few other trees of other varieties available for examination, the following account of the disease as it occurred on the Warner's King variety may be taken as describing a typical outbreak of blossom wilt.

The withered trusses had been carefully cut off from ten trees at one end of the row during the summer of 1915, while the rest of the trees in the row were subjected to the usual pruning operations only and many dead spurs and cankers, on which pustules of the fungus subsequently appeared, were left on the trees. That there would be another outbreak of the blossom wilt on these trees was expected therefore, and from the time the flowers began to open the trees were examined frequently for the first signs of the wilt, in order that the earlier stages of the disease could be recorded, as these had not been observed in 1915.

On May 1 it was noticed that a few of the flowers on these trees were open and the rest continued to open normally during the fortnight following. Nothing unusual was observed until May 17 when one truss was found to be withered and several others were showing the first signs of wilting as indicated by the flagging of the leaves at the base of each affected truss. This wilting of the leaves is the first evident symptom of the disease, for the flowers may fail to "set" and so wither from other causes.

From May 17 to the end of that month other trusses wilted and the following sequence was recorded for one tree:

Date	Affected trusses
May 17	2 trusses wilting (leaves beginning to flag)
,, 22	5 trusses dead: 2 others wilting
,, 27	18 trusses dead: 1 wilting
,, 31	19 trusses dead.

No trusses on this tree began to wilt after May 31.

In all cases where wilting of trusses occurred subsequently to May 31 they were found to be killed not by a direct infection through the blossom but by the development of the disease in the spur or branch from infection through flowers of other trusses. In several instances a spur bore two trusses one of which became infected directly and the fungus penetrated the tissues of the spur to the level of insertion of the un-infected truss which, in consequence of the interruption to the upward flow of sap to it, wilted from desiccation.

As an illustration of the rapid progress made by the fungus during the month of May the condition of a small branch which was completely girdled by the 31st of that month may be cited; on that day the shoots and trusses borne at the nine nodes commencing at the distal extremity were as follows:

Node 1. Vegetative shoot wilting (leaves yellow and drooping).
,, 2. Barren spur wilting.
,, 3. Dead truss with canker girdling the branch and extending 1 cm. upwards and 2·5 cm. downwards.
,, 4. Dead truss with canker nearly girdling branch.
,, 5. ,, ,, ,, halfway round.
,, 6. ,, ,, ,, nearly half round.
,, 7. Living healthy truss of flowers.
,, 8. Dead truss with canker just beginning.
,, 9. Living truss.

The trusses at nodes 3, 4, 5, 6 and 8 had been killed by direct infection while the shoots at nodes 1 and 2 were wilting by reason of the girdling canker at node 3; all the spurs were unbranched and about 1·5 cm. in length; the branch, where girdled, was 0·8 cm. in diameter.

As infection probably did not occur before May 1 it will be seen that in the course of about four weeks conidia had germinated and produced mycelium which had traversed the tissues of the flowers and the spurs and had invaded the branch itself to a distance of two and a half centimetres.

On the older parts of the branches (1·5 to 2 cm. in thickness) bearing short infected spurs to 2·5 cm. in length, there was evidence, towards the end of May, that the fungus was advancing beyond the level of insertion of the spurs, by the appearance of longitudinal fissures in the bark immediately above and below the nodes. By June 7 the affected areas on the branches were slightly sunken below the general surface and extended round the nodes as shallow elliptical depressions. Further extension of the fungus appeared to be inhibited about that date and although the margins of the cankers later became more defined, no further increase occurred superficially in the majority of those examined. The bark over the affected areas gradually became sulcate, often, on the smaller branches, with distinct "oyster-shell" markings of raised lines.

Cankers which had produced the *Monilia* pustules during 1915 had been labelled and all proved to be barren during 1916, no pustules being present; no further increase of the cankered surface had occurred and in each case callus was growing over it beneath the ruptured bark. Some cankers, several inches long, were almost completely healed (Fig. 7).

Thus on trees where the disease has existed for two or more years the following results are to be observed during the summer months:

A. Result of infection during that year:
  (1) dead trusses of blossom;
  (2) cankers on the branches round the bases of the shorter spurs bearing the wilted trusses.

B. Result of infection during the previous year:
  (1) dead spurs bearing *Monilia* pustules;
  (2) cankers also bearing *Monilia* pustules.

C. Result of infection two years previously:
  (1) dead spurs with rough cracked bark, not now bearing *Monilia* pustules;
  (2) cankers more or less healed over by callus growing over the cankered surface from the margin towards the middle line.

During winter affected trees are recognised by the dried remains of the infected trusses (see Fig. 1). Flowers which fail to "set" into fruit soon fall from the tree if the spur bearing them is not diseased. When affected by the blossom-wilt *Monilia*, however, they are retained on the tree through the winter. The leaves too, of these spurs, being killed before the absciss-layer is formed, remain attached to the tree.

In December 1916 the dead spurs and cankers caused by infection in

the spring of that year began to produce *Monilia* pustules which burst through the dead bark. In the following month these became somewhat pulverulent and particles of pustules placed in water set free mature conidia. On January 24, 1917, conidia were obtained in this way in a drop of water on a glass slide when the temperature of the air, as registered by a thermometer placed on one of the trees, was 28·5° F. (= – 2° C.). The slide was placed in a damp chamber at a temperature of 6° to 8° C.; within 24 hours about 50 % of the free conidia had germinated with germ tubes to 52μ in length.

This blossom-wilt fungus apparently occurs comparatively rarely on the apples themselves, even on trees severely infected with blossom wilt. On the apple trees at Wye it has been observed on the fruit on one occasion only when it was found on a few very young apples soon after they had set. In a plantation of Lord Derby trees in the Weald of Kent there were found in January 1916 small "mummified" apples nearly covered with a *Monilia* which in cultures was indistinguishable from that found on the cankers; in the same plantation in mid-July 1915 (and again in 1916) a few young apples, about 1·5 cm. in length, were obtained, apparently killed by the same fungus which appeared in the form of numerous pustules bursting through the skin. I have not yet seen this form on fully grown naturally infected apples.

(*c*) *Comparison with other diseases producing a similar condition.*

It is sometimes impossible to distinguish, from their general appearance, between the *Monilia* cankers and those produced by *Nectria ditissima*; the latter are usually recognised by the increased rate of growth (set up in the tissues bordering the canker) which causes the branch at that place to be swollen. Young *Nectria* cankers, also, generally bear the sporodochia of the *Fusarium* stage in the summer, or the pustules if not evident readily develop when placed in a damp chamber; young *Monilia* cankers do not produce pustules until about December. Again *Nectria* cankers originate at nodes bearing unopened buds, or infection occurs through wounds; those produced by the blossom-wilt fungus arise around the insertion of infected flowering spurs.

The blossom-wilt condition bears a close resemblance to that caused by the Apple Sucker (*Psylla mali*); when the damage is caused by this insect, however, numerous cast skins are to be found attached to the under side of the withered leaves. The larva of the Pith Moth also produces a withering of the fruit buds but before the flowers themselves

expand, while in the blossom wilt caused by the fungus the effect is not noticeable until about a fortnight after the flowers open.

The disease has by some growers been attributed to late frosts; inoculation experiments (described later in this paper) carried out in a greenhouse show conclusively that the fungus readily produces the wilt in the absence of frost.

It would seem that the diseased twigs and branches bear a close resemblance to those attacked by "Fire-Blight" (*Bacillus amylovorus*) in America. Prof. H. H. Whetzel, Professor of Plant Pathology in the Cornell University, U.S.A., when he visited Wye College a few years ago, saw trees badly cankered by *Monilia*, and informed us that the general appearance of the diseased branches was indistinguishable from the condition induced by the attacks of the *Bacillus*, and, as already pointed out, Aderhold at first attributed the "Zweigdürre" of apple trees to the "Fire-Blight" organism. In this connection it may be mentioned that dead apple twigs examined by Prof. Worthington E. Smith (20) during the winter of 1898–9 were pronounced by him to have been killed by "Fire-Blight" since bacteria were found in the dead tissues. No conclusive evidence that *Bacillus amylovorus* occurs in this country has yet been published, and as old spurs killed by *Monilia* often contain bacteria in abundance, it seems probable that Prof. W. E. Smith was mistaken in his diagnosis and that the specimens seen by him were twigs killed by Brown Rot.

IV. The Blossom Wilt Fungus compared with other *Monilias* of Fruit Trees.

(a) *Cultural studies of Monilias found in this country.*

While circumstantial evidence obtained during observations made in the open made it very probable that the *Monilia* found on the spurs and cankers produced infection of the flowers, for confirmation of this it was necessary to reproduce the disease by the inoculation of flowers, using pure cultures. The organism has therefore been cultivated on sterilised media, and the cultures obtained have provided a means of comparing this with other forms of *Monilia* with respect to certain characters appreciable only in pure cultures.

Some thirty strains of the fungus have been cultivated in pure culture, many of them in duplicate, and, without exception, no constant differences could be detected in the various strains. Two methods of obtaining the cultures have been adopted. When the fungus is found in the

conidial stage, conidia are isolated on agar plates; the resulting sporelings which are seen to be uncontaminated are transferred to other plates and when sufficient growth has taken place sub-cultures are prepared from these. Cultures for the continued propagation of any particular strain are made on agar slants in test tubes. Strains have been obtained in this way from dead spurs, cankers and young apples; one was prepared from conidia induced on a dead truss by placing it in a damp chamber, and another from a "mummied" apple.

When the fungus is in the form of sterile mycelium only, as in the majority of freshly killed spurs and young cankers, the surface of the affected parts is gently rubbed over with cotton wool damped with absolute alcohol[1], and transverse sections are made through the diseased tissues with a sterilised razor. These are dropped into a watch-glass containing sterile distilled water and particles of the sections are then removed with flamed needles (avoiding the outer layers of the cortex) to another watch-glass of sterile water, from which they are finally transferred to agar plates. As a rule cultures which were apparently quite pure could be obtained directly in this way; sometimes, particularly in the case of spurs which had been dead for some weeks, bacterial colonies appeared immediately round the section but sub-cultures taken from the peripheral mycelium could be obtained pure. Such cultures were indistinguishable from those obtained from conidia.

For comparison with these, cultures of the Brown Rot fungi from other sources have also been prepared, e.g. forms growing on various "stone-fruits" (plum, cherry, damson, peach), on apples and on pears. Each of the strains has been started from a single conidium. In those instances where no viable conidia were present this could not be done directly and the cultures were obtained initially from sterile hyphae; the production of conidia was induced by growing on sterilised potato, and "single-spore" strains were then prepared.

I have not yet obtained the ascigerous (*Sclerotinia*) stage of any of these forms, so have been unable to compare them with the descriptions of that stage as found on the Continent and in America. The fungus which appears so generally on ripening apples is however undoubtedly the *M. fructigena* as described in Woronin's paper (23), from the large size of its pustules (usually 1·5 to 2 mm. in diameter), the colour of the pustules, which is a buff yellow, and the large size of the conidia; the latter are usually about 20μ in length but often exceed that. This species I have obtained from the apple, pear, cherry, plum and peach,

[1] 94 % alcohol has also been used with equally good results.

but, to the time of writing, only on the fruit and not on the flowers or twigs[1].

The blossom-wilt *Monilia*, as it occurs on the twigs, fruiting spurs and cankers of the apple tree in winter and spring, has pustules generally smaller (but they may reach a diameter of 1·5 mm.) and grey in colour rather than yellow, though when very powdery they may become somewhat ochraceous and are then easily mistaken for *M. fructigena*, while the conidia are much smaller, being usually 10–12μ in length and rarely exceeding 14μ. When, as occasionally happens, it occurs on young apples in summer the dimensions of the conidia are greater and this again may lead to some confusion.

The two species grow vigorously on suitable sterilised culture media and the utilisation of this property will probably afford a ready and more definite means of identifying them, particularly as cultural methods can be employed when the fungi are in the condition of sterile mycelium only. Differences in habit of growth and development of conidia distinguish them when growing on two easily prepared media, viz., prune-juice agar and steamed potato.

The medium generally used by the writer to obtain mycelial growth is a decoction of prunes (1 % stoned prunes) containing 1·5 or 2 % agar-agar. *M. fructigena* produced, on plate cultures of this medium, a flat disc of mycelium, circular in outline and growing out regularly to the edge of the plate with a margin laciniate or almost entire. The growth of the blossom-wilt *Monilia* at first resembles that of *M. fructigena* but soon shows a tendency to become lobed, the lobes being deltoid or flabelliform with narrow sinuses between them; a characteristic feature of these cultures is for growth to be arrested at about mid-way between the centre and the edge of the plate and fresh growth commences as flabelliform radiations from between the lobes. This condition has not been observed in cultures of *M. fructigena*.

Neither species produces conidia on the prune-juice agar medium although both give rise to numerous clusters of minute spore-like bodies produced in radiating chains which in the earlier stages of development resemble the fructifications of a *Penicillium* but later become dense irregular clusters. These "sporidia" were observed on *M. fructigena* by Humphrey (11) who states that they germinate and produce mycelium. Woronin figures them for *M. fructigena* and *M. cinerea* but failed to

[1] Since the present paper was sent to the press evidence has been obtained which suggests that *M. fructigena* may extend from the infected fruit into the branches and form cankers, in the case of certain varieties of soft-wooded apple trees (e.g. James Grieve); this point is being investigated.

induce them to germinate; attempts by the present writer to obtain germ tubes from these sporidia have also failed.

Of sterilised media the most suitable for the development of conidia has hitherto proved to be steamed potato, and in diagnosing a fresh strain this is at present always used. Roux's tubes are found useful for these cultures though ordinary wide test tubes are also employed. Vigorously growing mycelium transferred from an agar plate will reach the edge of the potato in four days and tufts of conidia are produced within a week. Measurements of conidia from potato cultures were taken when the cultures were 6–8 days old; if left for a longer period the conidia, particularly those of the canker strains, became overgrown by hyphae probably from their own germ tubes. The conidial tufts of *M. fructigena* in these cultures are yellow in colour and usually form distinct continuous raised zones towards the upper end of the potato, while those of the canker strains were smaller, scattered, more diffuse, and grey in colour.

An examination of the *Monilias* found on the stone-fruit trees (*Prunus* spp.) in this country led to the discovery that there are (in addition to *M. fructigena* which occurs frequently on the plum and sweet cherry) two forms both of which may be referred to *M. cinerea*, though they differ from one another culturally, and one of these appears to be identical with that form which causes the destruction of the apple blossom. The latter when growing as plate-cultures on prune juice agar is at first hyaline, but, when growth has extended for about 2 cm. from the point of inoculation, a circular band appears, olive green to dark brown in colour, approximately at 1 cm. from the centre, and later other brown zones develop. This brown coloration is more intense when the fungus is grown as agar slant cultures and appears to be favoured by the depth of the medium, for the brown zone first appears as a curved transverse band on the lower (deeper) side of the point of inoculation. The colour becomes darker and more general with the age of the culture which eventually is almost black throughout. Strains which also become brown on this medium have been obtained from the stone-fruit but whether any of these are able to infect the apple flower has not yet been determined.

Other strains, obtained from plums and sweet cherries, remain hyaline both in plate and tube cultures of prune-juice agar. Of two strains obtained by isolating conidia from a damson, one retained the hyaline appearance, the other produced a dark brown band when the two were grown side by side on the same plate.

Other differences have also been detected between the two types; thus the blossom-wilt form produces conidia less freely than the hyaline form from plums when grown on steamed potato. Again when inoculations are made on mature apples the former soon causes the fruit to become dark brown to black with conidial pustules absent or very few in number, while with the latter the dark colour is less pronounced and numerous pustules are readily developed.

Four strains have been obtained from flowers and twigs of acid (Morello) cherry trees and these on prune-juice agar produce a brown coloration which however is not so uniform as that of the strains from the apple trees.

Whether the hyaline form is less virulent than the brown is at present uncertain, and it is proposed to carry out inoculation experiments with that object in view, but it is significant that all the strains obtained from wilted trusses and cankers of the apple tree produce on the agar cultures a coloration which eventually becomes almost black.

(*b*) *Dimensions of Conidia.*

Apart from the colour of the pustules, the size of the conidia is the character most generally quoted as determining the species of *Monilia* (in the absence of the ascigerous stage) found on cultivated fruit. Of the strains of the blossom-wilt fungus at first studied only a few conidia were measured; it was soon evident however that not only did the average size of the conidia vary with the conditions under which they were produced, but also that there was considerable variation in the dimensions of conidia taken from the same pustule. The number taken therefore in order to obtain a fair average was in each case 100, for those strains obtained more recently.

It has already been stated that the *Monilia* of the apple blossom wilt has in general smaller conidia than *M. fructigena* and this difference is particularly well marked if a comparison is made of the dimensions in the two forms when each is producing pustules most abundantly under natural conditions. Thus if conidia of the blossom-wilt fungus are taken from cankers or spurs in the spring when this form is most conspicuous, the typical conidia are found to measure $11–12 \times 8–9\mu$. *M. fructigena* on the other hand is most vigorous in late summer and early autumn when the majority of its conidia reach or exceed 20μ in length. If, however, the two are grown under the same conditions, as, for example, on sterilised potato in the laboratory the difference is less

striking, for the conidia of the blossom-wilt *Monilia* are then considerably larger than when they are produced on spurs and cankers.

Four strains obtained from dead spurs and cankers from February to April, 1916, were subsequently grown on steamed potato and in each case 100 conidia were measured[1] with the result as shown in the table. The conidia taken from the sterilised potato were measured when the cultures were 6–8 days old.

Strains of the Blossom Wilt *Monilia* from spurs and cankers of apple trees	Average of 100 conidia taken from spurs or cankers	Average of 100 conidia taken from cultures on steamed potato
Strain XI from dead spur, Feb. 1916	$12{\cdot}0 \times 8{\cdot}5\,\mu$	$19{\cdot}0 \times 15{\cdot}0\,\mu$
,, XII from dead spur, March 1916	$11{\cdot}5 \times 8{\cdot}5\,\mu$	$19{\cdot}0 \times 15{\cdot}5\,\mu$
,, XIII from a canker, April 1916	$11{\cdot}5 \times 8{\cdot}5\,\mu$	$18{\cdot}0 \times 14\,\mu$
,, XIV from another canker, April 1916	$11{\cdot}0 \times 8{\cdot}0\,\mu$	$18{\cdot}0 \times 13{\cdot}5\,\mu$

Strain XIV was subsequently used in inoculations on apple blossom in the open, and conidia obtained from an infected flower stalk in July had an average size (for 100 conidia) of $15{\cdot}5 \times 12{\cdot}0\,\mu$, but conidia taken during the following winter (February 1917) from a spur killed by inoculation with this same strain gave an average of $11{\cdot}0 \times 8{\cdot}5\,\mu$ which is a close approximation to that obtained from conidia taken from the original canker.

A strain obtained from young apples soon after setting (June 12, 1915) was found to have conidia the majority of which were from 17 to $20\,\mu$ in length but sufficient measurements were not made for an average to be taken comparable with the above. Another strain obtained from young apples in July 1916 gave an average of $18 \times 13\,\mu$ for 100 conidia taken from the fruit itself, and when grown on steamed potato the average was about the same, i.e. $17{\cdot}5 \times 14{\cdot}0\,\mu$. The other cultural characters of these two strains, e.g. habit of growth and brown coloration on prune-juice agar, and grey conidial tufts on potato, resemble those of the typical blossom-wilt strains.

It has not yet been ascertained what factors determine the output and the size of the conidia, but probably temperature and the degree of moisture have some influence. The conidia are invariably smallest if obtained in winter when the fungus is living on dead wood and bark; they are much larger when produced on young apples in the summer or on potato at the temperature of the laboratory (15°–18° C.).

Therefore when a *Monilia* obtained from an apple tree is examined with a view to its identification it is essential that the conditions under

[1] The readings were taken correct to $0{\cdot}5\,\mu$ and the averages were calculated also correct to the nearest $0{\cdot}5\,\mu$.

which the conidia are produced be taken into consideration. The range of variation and the average size of the conidia of the blossom-wilt fungus under certain conditions is indicated in the accompanying table:

Dimensions of the Conidia of the Blossom-Wilt Monilia.

Source of conidia	Range of variation	Average
Spurs and cankers in winter and spring	7 × 5 to 20 × 13 μ (usually 10–14 × 7–9·5 μ)	11·5 × 8·5 μ
Young apples in the open in summer (June and July)	9 × 7·5 to 25 × 17 μ (usually 16–20 × 11–14·5 μ)	18 × 13 μ
Potato cultures at temperature of laboratory (15°–18° C.)	9 × 7 to 26 × 20 μ (usually 16–20 × 11–14·5 μ)	18·5 × 14·5 μ

When this fungus is growing on young apples in the open or on steamed potato in the laboratory the dimensions of the conidia approach those of *M. fructigena*, but the smaller size and grey colour of the pustules distinguish it from the latter.

Strains of grey *Monilias* on plums show a similar variation as here shown:

Strains of grey Monilias from plums	Average of 100 conidia from original source of strain	Average of 100 conidia taken from culture on steamed potato
Strain VII, mummied plum, February	11·5 × 8·0 μ	16·0 × 12·5 μ
,, VIII ,, ,, April	11·5 × 8·0 μ	16·0 × 12·0 μ
,, IX ,, ,, April	12·0 × 8·0 μ	17·0 × 12·0 μ
,, X, dead twig, June	14·0 × 10·5 μ	16·5 × 12·0 μ
,, XI, young green plum, June	16·0 × 10·0 μ	16·0 × 12·0 μ
,, XII, canker on plum tree, August	no pustules present	15·5 × 11·5 μ
,, XIII, mature plums, August	14·5 × 11·0 μ	15·5 × 11·5 μ

Strain VIII on prune-juice agar produced a browning of the medium comparable with that caused by the blossom-wilt form of apples; the others remained hyaline except Strains X and XI which were intermediate in this respect, the coloration being less pronounced (hardly perceptible in plate cultures) than in Strain VIII.

These strains from plums generally produced conidia more readily on steamed potato than did those from apple spurs and cankers; the conidial tufts of the latter were always scanty whereas the former may produce definite concentric circles of conidiophores.

Four strains have been isolated from Morello (acid) cherries and though they have not yet been closely studied they behave in cultures as the Strains X and XI from plums.

Monilia fructigena shows less variation in the size of its conidia but I have not yet found this species producing fresh chains of conidia in

the winter time; pustules examined in March and April have been barren or the conidia present were not viable, a condition observed on the Continent by Ewert (9). Three strains of *M. fructigena* of which a sufficient number of conidia have been measured gave dimensions as follows:

Strains of *M. fructigena*		Average of 100 conidia from original source of strain	Average of 100 conidia taken from cultures on steamed potato
Strain VII, mummied apple, June	...	19·0 × 12·5 μ	19·5 × 11·5 μ
,, VIII, young plums, July	...	21·5 × 13·0 μ	20·5 × 13·0 μ
,, IX, mature apple, October	...	20·5 × 13·5 μ	20·0 × 12·5 μ

(*c*) *American Strains of Monilia.*

As much attention has been given to the study of Brown Rot diseases in America it was possible to obtain specimens and cultures from that continent for comparison with those described in the preceding pages. In all, ten strains have been cultivated and examined. Apples and cultures were received from Dr G. B. Posey of the Oregon Agricultural College, a culture (from a peach) and mummified plums from Prof. L. R. Jones, Professor of Plant Pathology in the University of Wisconsin, mummified plums and peaches, together with cultures prepared from ascospores, from Mr W. A. McCubbin, Laboratory of Plant Pathology, Ontario. Thus the ten strains were from three different hosts, viz. apples, plums, and peaches, and represented three widely separated regions.

The strains are characterised by certain features common to all of them but absent from the *Monilias* obtained from material collected in this country. The most striking character peculiar to the American form is the readiness with which it produces conidia on sterilised culture media; even on prune-juice agar, as plate or as tube cultures, numerous tufts of conidiophores develop, usually within three or four days from inoculation.

When growing on plates of prune-juice agar the mycelium grows out to form a regular circular disc with an entire or sub-entire margin, resembling the growth of *M. fructigena* in its rate of development and general habit rather than that of our grey *Monilias*; the production of numerous tufts of conidiophores (often in concentric zones) in these cultures serve to distinguish it from *M. fructigena.*

On steamed potato the conidial tufts are grey and are usually so numerous that they form an almost continuous pulverulent layer over the surface. In these cultures therefore the general appearance of the

American form is very unlike that of *M. fructigena* and the conidia too are smaller than in the latter as will be seen by comparing the dimensions given below with those of *M. fructigena*.

The dimensions of the conidia of those strains where 100 conidia were measured were as follows:

	Average of 100 conidia		
	From the fruit	Agar culture	Potato culture
Strain C, mummied peach from Ontario	$16{\cdot}5 \times 12{\cdot}0\,\mu$	$14{\cdot}0 \times 9{\cdot}5\,\mu$	$16{\cdot}0 \times 12{\cdot}0\,\mu$
,, D ,, plum ,, ,,	$17{\cdot}0 \times 12{\cdot}5\,\mu$	$14{\cdot}0 \times 9{\cdot}5\,\mu$	$16{\cdot}0 \times 12{\cdot}5\,\mu$
,, E, culture (peach) from Wisconsin	—	$14{\cdot}0 \times 9{\cdot}5\,\mu$	$15{\cdot}5 \times 10{\cdot}5\,\mu$
,, F, mummied plum ,, ,,	No suitable conidia present	$14{\cdot}0 \times 9{\cdot}0\,\mu$	$16{\cdot}5 \times 11{\cdot}0\,\mu$
,, G, ascosporic culture (peach) from Ontario	—	$14{\cdot}0 \times 9{\cdot}5\,\mu$	$14{\cdot}0 \times 10{\cdot}5\,\mu$
,, H ,, ,, (plum) ,, ,,	—	$14{\cdot}0 \times 8{\cdot}0\,\mu$	$13{\cdot}5 \times 10{\cdot}0\,\mu$

The most uniform results are obtained on prune-juice agar but as all the British strains do not develop conidia[1] under those conditions a comparison of the size of the conidia of the various forms from this culture medium cannot be made.

Growth is vigorous on the media employed; in one case conidia placed on agar germinated at room temperature (about 18° C.) producing within three days sporelings 3 mm. in diameter, and already short chains of conidia were present. On prune-juice agar plates growth takes place at a more rapid rate than in the case of the grey *Monilias* of this country, and the periodicity in rate of growth observed in the latter is absent from the American form which grows out uniformly from the point of inoculation to the edge of the plate.

In a paper recently published Bartram (5), working with strains of *Monilia* obtained in Vermont, concludes that the form in that state is *M. cinerea* but finds that it produces conidia in agar cultures; it is probable therefore that the Vermont strains resemble those received at Wye from Oregon, Wisconsin, and Ontario and that that form is the one commonly found in North America.

(*d*) *Continental Strains of Monilia.*

From our geographical situation and the frequency with which nursery stock is imported from the Continent it is natural to expect that the Monilias of this country would be closely related to, if not identical with, those of the rest of Europe, and from descriptions

[1] Very rarely have I seen an occasional short chain of conidia on plate cultures of *M. fructigena* on this medium, but no definite pustules or tufts of conidiophores have developed, nor sufficient conidia for comparative measurements.

available it seemed probable that *M. fructigena* and *M. cinerea* of the Continent were present on our own fruit trees. When it was found that cultural methods were a great aid not only in distinguishing *M. fructigena* from the grey *Monilias*, but could also be employed for the recognition of certain forms among the latter, efforts were made to obtain strains of the Brown Rot fungi from the Continent.

To the time of writing I have only succeeded in obtaining specimens from Holland, Dr H. M. Quanjer of the Instituut voor Phytopathologie, Wageningen, having on several occasions kindly forwarded mummified fruit. The fungus usually present on such material was a yellow one, in cultures indistinguishable from the yellow form of England, and there can be no doubt that this is *Monilia fructigena* Pers. One strain from these specimens was obtained from a mummified pear received in March 1916. Conidia were fairly numerous and on the average measured $19{\cdot}5 \times 9{\cdot}5\,\mu$, dimensions which are greater than the average size of conidia from grey *Monilias* in winter; these conidia failed to germinate when placed on agar so evidently they had not survived the winter. Particles of a pustule were teased out in sterilised water and placed on a prune agar plate; these grew out but were impure. More successful results were obtained by cleansing a portion of the skin with cotton-wool soaked with 94 % alcohol, then, raising a portion of this sterile skin with a scalpel, particles of the pulp were removed and placed on an agar plate. The resulting culture was apparently pure; sub-cultures on potato yielded conidia and the isolation of some of these afforded pure sporelings. Sub-cultures from the sporelings were then grown for comparison with other strains of *M. fructigena* and no appreciable difference has been detected either in the mode of growth or coloration; on potato the average size of the conidia was $21{\cdot}0 \times 12{\cdot}5\,\mu$.

One batch of fruit from Dr Quanjer received early in January 1917 included plums bearing grey *Monilia* pustules with viable conidia and a strain was isolated from each of two of the plums. Conidia, taken from the plums as received, and later obtained from potato cultures, were measured and their dimensions found to be as follows:

	Average of 100 conidia in each case	
	From mummied plums	From potato culture
Grey *Monilia* from Holland, Strain 1	$11{\cdot}5 \times 8\,\mu$	$16{\cdot}5 \times 12{\cdot}5\,\mu$
,, ,, ,, ,, 2	$11{\cdot}0 \times 7\,\mu$	$16{\cdot}0 \times 12{\cdot}0\,\mu$

It will be seen that these figures approximate closely to those obtained, under corresponding conditions, from strains found on plums grown in

this country. The mode of growth on culture media is also similar to that of our grey *Monilias*. Neither of the strains from Holland has produced definite brown zones in tube cultures of prune-juice agar but irregular brown patches eventually appeared. They thus resemble (and are probably identical with) a form frequently found on plums and acid cherries in Kent.

I have not yet obtained diseased apple twigs from abroad.

(*e*) *Nomenclature of the various forms.*

In cultures it is possible therefore to distinguish four types of *Monilia* as found on cultivated fruit trees of the genera *Pyrus* and *Prunus*, as here tabulated:

	Prune-juice agar plate cultures	Cultures on steamed potato in Roux' tubes
(1) *Monilia fructigena*, occurring commonly on apples and plums, and frequently on sweet cherries.	Margin almost entire or laciniate; no brown coloration; conidia absent.	Conidial tufts yellow, well-developed at upper end of potato, forming raised zones.
(2) Blossom Wilt *Monilia* of the apple, also occurring occasionally on plums.	Margin with deltoid or flabelliform lobes, growth usually arrested about mid-way between centre and side of plate, and new outgrowths as flabelliform lobes develop, usually from the sinuses; olive-green, to brown, zones appear, the first usually at 0·5 to 1 cm. from the centre; conidia absent.	Conidial tufts grey, few and scattered.
(3) A grey *Monilia* frequent on plums and sweet cherries.	As above but no brown zones appear.	Conidial tufts grey, more numerous than in (2), often appearing in concentric circles round point of inoculation.
(4) American form of *Monilia*.	Margin entire or crenate; conidial tufts numerous, usually in concentric circles; brown coloration of the agar absent or appears as a peripheral band near edge of plate; growth generally more rapid than in (2) or (3) and more uniform.	Conidial tufts grey, almost covering the whole surface in a continuous layer.

As shown in the table the successive brown zones typical of the plate cultures of the blossom-wilt *Monilia* do not appear in cultures of the other forms; sometimes a brown coloration occurs in the plate cultures of the American form but in that case it commences as a peripheral band when the mycelium approaches, or has reached, the edge of the

plate. When growing as "slant cultures" in test-tubes both *M. fructigena* and the American *Monilia* usually produce a browning in old cultures, but this commences at the extreme lower end of the slant and gradually extends for some distance upwards, while in the case of the blossom-wilt fungus the first brown zone appears usually from 0·5 to 1 cm. from the point of inoculation when the culture is still quite young (often within a week).

The typical hyaline form from plums and sweet cherries remains colourless even in old slant cultures.

On the Continent, opinion is divided as to whether all instances of Brown Rot, caused by *Monilia*, on fruit trees are to be attributed to one species only (retaining for it the name *Monilia fructigena*), or to more than one. Those who favour the latter view generally assume that there are three species concerned, viz. *M. fructigena*, said to occur chiefly on the pomaceous fruits, characterised by its yellow pustules and comparatively large conidia which usually are not viable in winter: *M. cinerea*, considered to be found almost exclusively on the "stone" fruits, with grey pustules and smaller conidia which retain viability throughout the winter: and *M. laxa*, a grey form, with small conidia, occurring on apricots. Aderhold and Ruhland (4) claim to have proved that these three species are to be distinguished by their respective ascigerous (*Sclerotinia*) stages, which they describe. *S. fructigena* they obtained from mummified apples, and from the ascospores were able to develop the yellow *Monilia* stage, *M. fructigena*. That the two forms are stages in the life-history of one fungus appears therefore to be fully established. With regard to *Sclerotinia cinerea* their conclusions are less convincing. They were unable to obtain an ascigerous form from peaches, plums or cherries, so described the form, which they refer to *S. cinerea*, from apothecia (preserved in spirit) which had been found on mummified peaches and had been sent to them from America, and assumed that the *Monilia cinerea* of Europe is its conidial stage. Mummified apricots yielded a *Sclerotinia* which they described and named *S. laxa*, with *Monilia laxa* as its conidial stage.

Since, as has been shown above, the *Sclerotinia* generally occurring in America produces a conidial stage which can be readily distinguished, in cultures, not only from the grey *Monilias* of this country but also from those strains obtained by the writer from plums received from Holland, it would seem probable that Aderhold and Ruhland included in their diagnosis of the one species *Sclerotinia cinerea* two forms which are very different when grown under certain cultural conditions.

The description given of *Monilia laxa* of the apricot, as the conidial stage of *Sclerotinia laxa* applies equally well to the grey *Monilias* of other fruit. The most striking difference they give is in the size of the conidia, viz. *M. cinerea* $9{\cdot}3$–$14{\cdot}5 \times 6{\cdot}2$–$12{\cdot}4\,\mu$, *M. laxa* $12{\cdot}4$–$23{\cdot}8 \times 9{\cdot}3$–$15{\cdot}5\,\mu$, but this difference is no greater than that shown by the blossom-wilt fungus or the Monilias of plums when growing under such diverse natural conditions as (*a*) mummified fruit or dead spurs in winter, (*b*) fresh fruit in summer.

The present writer is of the opinion from the evidence to hand that the grey *Monilias* of this country are to be referred to the continental form *M. cinerea* Bon., the *Sclerotinia* stage of which has not yet been described, unless indeed it should prove to be identical with *Sclerotinia laxa* (Ehrenb.) Aderh. et Ruhl. The blossom-wilt fungus must provisionally therefore be included under *Monilia cinerea* Bon. This species as at present delimited includes at least two forms, one which remains colourless in prune-juice agar cultures, and another which produces zones of an olive green to dark brown colour. The American Brown Rot fungus appears to be a distinct form and it may be necessary to distinguish it specifically from the European species.

V. Inoculation Experiments with Pure Cultures.

As observations in the open had indicated that in all probability infection occurred through the open flower, it was necessary that inoculation experiments should be carried out when the trees were in bloom, and the time available under natural conditions was thus limited to about a fortnight in the year. It was decided therefore that a first series of inoculation should be performed under glass in order that any information thus obtained, relative to the mode of infection, could be utilised during the same spring in subsequent experiments in the plantation. Another reason for conducting the experiment under these conditions was to eliminate the possibility that the action of frost would modify the course of the experiment.

Some difficulty was experienced in obtaining conidia in sufficient quantity to ensure successful inoculation. With the object of inducing the fungus to produce conidia readily on a sterilised substratum many culture media were tried, e.g. agar-agar media: $1{\cdot}5\ \%$ agar in an extract of each of the following substances: celery, prunes, French beans, apple twigs, malt culms (also malt culms + $10\ \%$ starch), maize meal, potato. Prune-juice agar containing strips of filter paper was also used.

Woronin obtained conidia of *Monilia cinerea* on gelatine cultures prepared with apple juice or bread broth; these were tried for the apple blossom *Monilia* but without success. Nor were conidia produced on bread steamed in test tubes or on pieces of apple branches sterilised in the autoclave. The best results were obtained with semi-cylinders of tubers and roots as potato, artichoke (*Helianthus tuberosus*), carrot, mangel and parsnip. The last two produced a trace of conidia, the other three developed conidiophores in scattered tufts, and potato, sterilised either by intermittent steaming (20 minutes at 100° C. for each of three successive days) or in the autoclave for 20 minutes at 115° C., was generally used as the medium for cultures used in the inoculation of apple flowers.

(*a*) *Inoculation of apple flowers in the Greenhouse.*

For this experiment young apple trees about three feet in height were acquired; for convenience they were planted in pots early in the year (1916) and left outside until the fruit buds showed the first signs of expanding. The pots were then transferred to the greenhouse, with the result that the earliest flowers were fully expanded during the first week in April.

Conidia for the inoculations were taken from cultures, about seven days old, on steamed potato (in one instance a culture on steamed carrot was used). They were removed from the culture tube and placed on the stigmas of the flower by means of a sterilised platinum needle, which was re-sterilised in the flame of a spirit lamp after each inoculation. One flower was successfully inoculated by inserting the needle between the stamens and the styles so that the conidia were deposited at the base of the styles, but this method was not generally adopted as usually there was no space between the filaments for the insertion of conidia within the hollow receptacle of the flower.

One flower only of each inflorescence was inoculated, and of twenty-four flowers thus treated thirteen not only withered but produced a wilting of the trusses to which they belonged. The results obtained on the four varieties tested were as here shown; in one column appears

Variety	No. of flowers inoculated	No. of successful inoculations
Prince Bismarck	9	7
Lord Derby	4	2
Cox's Orange Pippin	5	2
Worcester Pearmain	6	2
Totals	24	13

the number of trusses treated (i.e. one flower of each inoculated), and in the other is the number of successful inoculations resulting in each case in the death of the whole truss.

The blossoms of the Prince Bismarck trees were the first to expand and the earliest of these were inoculated immediately after opening, some of them on the first day on which the stigmas were exposed. The flowers of the other varieties were as a rule rather older when inoculated and this suggests (judging from results as indicated in the table) that the flowers are most susceptible to infection immediately after they open. None of the untreated trusses showed any signs of wilting throughout the season except in those cases where the fungus had extended from the inoculated flowers so far as to cut off supplies to those parts not directly infected.

The results obtained from these experiments, in those instances where inoculation of a flower was followed by the wilting of the remaining flowers and also the leaves of the spur, are given below in detail to illustrate the progress of the disease from the time conidia were placed in the flowers.

All the trees were removed from the greenhouse about the middle of May and were left in the open throughout the winter.

Variety *Prince Bismarck*.

(*a*) Flower of a spur 1 cm. in length inoculated April 4 by placing conidia on the stigmas. On April 11 the styles were dark brown for a distance of 4 mm.; the whole truss was dead before the end of April and on May 5 a canker extended from the base of the spur to three-quarters of the distance round the branch.

(*b*) Flower of a spur 2·5 cm. long inoculated April 8; another truss of flowers borne on the same spur was untreated. The inoculated truss was dead by April 26 and the disease had reached the base of the other truss on that spur so that this also wilted.

(*c*) and (*d*) Flowers inoculated April 8. Both trusses were dead on April 26.

The trusses thus treated were all borne on a branch bearing in all six trusses of flowers, four of which were infected directly as shown; the fifth became infected indirectly from truss (*b*), and the sixth also wilted early in May as the result of a canker produced at the base of the inoculated truss (*d*) at the next node below. The cankers developing round the bases of the infected spurs later became confluent and by June 8 that portion of the branch bearing the trusses was cankered for

a distance of 19 cm. or from 3 cm. above truss (*a*) to 7 cm. below (*d*); the terminal portion of the branch died as a result.

The fungus itself was not observed until the first week in December when young pustules with chains of immature conidia (the conidia remained cohering in chains when particles of the pustules were mounted in water) were seen at the lower end of the canker. Later others appeared and by Jan. 9 numerous conspicuous pustules were present along the whole length of the canker and on the infected spurs. By this time the conidia were more or less pulverulent as a considerable number became free on mounting in water; such conidia were viable and many of them germinated within forty-eight hours when the slide was kept in a damp chamber at a temperature of 6° to 8° C.

(*e*) On another branch a truss was infected by placing conidia at the base of the styles of one flower on April 8. The truss wilted before the end of the month and a canker developed which had nearly girdled the branch when examined on June 8. Immature *Monilia* pustules were present at the base of the spur on Dec. 6; these were well developed by Jan. 9.

(*f*) On the same branch as (*e*) a similar truss was infected by placing conidia on the stigmas of a flower. The spur was killed before April 26, but no canker was produced on the branch. One *Monilia* pustule was found on the spur on Jan. 9.

(*g*) A truss inoculated on April 11 was dead within fifteen days. On April 27 the twig bearing it was removed from the tree and it was found that the tissues of the spur (which was 1 cm. in length) were brown to the level of its insertion on the twig and the disease was already encroaching on the tissues of the twig itself. In sections made transversely through the spur there were found numerous hyphae, particularly in the pith, and particles of the sections placed on an agar plate produced mycelial growth resembling that obtained in a similar manner from naturally infected spurs.

The exact date when the trusses of this variety first showed signs of wilting was not ascertained, but all those referred to above were dead on April 26 and had probably begun to wilt some days previously. The varieties which were treated at a later date were examined more frequently and the earlier symptoms of the disease noted.

Variety *Cox's Orange Pippin.*

(*a*) Truss of a spur 1 cm. long on a branch (0·4 cm. in thickness) bearing a terminal vegetative shoot distal to the inoculated truss.

April 19. A single flower inoculated by placing conidia on the stigmas.

„ 27. Stigmas brown to base, stamens drooping.

May 1. Truss wilting: flowers and leaves drooping, the latter curled and brown.

„ 5. Branch, bearing the truss, half girdled by a canker proceeding from the base of the spur.

„ 9. Branch three-fourths girdled: leaves of the vegetative shoot on the same branch flagging.

June 8. Canker extending to 5 cm. below the infected spur. The upper limit of the canker was not distinctly marked, but the distal portion of the branch was quite dead by this time.

Jan. 9 (1917). Well-developed *Monilia* pustules present on the dead spur.

(*b*) Truss of a spur 0·5 cm. long on a twig (0·4 cm. in diameter) bearing another flowering spur distally.

April 19. A single flower inoculated as in (*a*).

„ 27. Stigmas of inoculated flower brown and withered, stamens collapsed, pedicel discoloured to its base, sepals brown and withered. Other flowers of the same and of the neighbouring trusses showed a little browning of the stigmas on this date but in every case the stamens were upright and the pedicels and sepals were not discoloured.

May 1. Truss withered: flowers drooping, leaves curled and brown.

„ 3. The other truss (not inoculated) on same twig wilting.

„ 5. Twig completely girdled, both trusses dead.

June 8. Twig dead to the level of its insertion on the stem, but no canker had developed on the latter.

Jan. 9 (1917). One *Monilia* pustule present at the base of the spur.

(*c*) A flower inoculated on April 19 showed the same symptoms of the disease as in (*a*) and (*b*) to April 27 when the stigmas were brown to the base, the stamens collapsed and the pedicel brown throughout its length; on April 29 however it was accidentally removed, evidently before the fungus had invaded the tissues of the spur itself for the leaves were still alive in June. None of the flowers of this truss set into fruit but this was probably due to non-pollination.

Variety *Worcester Pearmain.*

(*a*) Truss of a spur 1 cm. in length on a branch 0·4 cm. in diameter.

April 19. One flower inoculated on the stigmas.

,, 27. Styles brown to the base, stamens collapsed. On a neighbouring control truss of the same age the styles were not brown and the stamens were upright.

,, 29. Flowers and leaves of the truss drooping.

May 1. Truss withered, leaves brown and curled.

June 8. Canker present on the branch, nearly girdling.

Dec. 6. Young pustules (bearing very short chains of immature conidia) appearing.

Jan. 9 (1917). Conspicuous *Monilia* pustules present on the canker and dead spur.

(*b*) Truss of a spur 1·5 cm. in length on a branch 0·5 cm. in diameter.

April 19. One flower inoculated on the stigmas.

,, 27. Styles of inoculated flower brown, some of the stamens collapsed; these features were not present on a neighbouring control truss.

,, 29. Flowers and leaves of the truss drooping.

May 1. Truss withered, leaves brown and curled.

June 8. Canker present round the base of the spur, girdling the branch and extending 2·5 cm. below the insertion of the spur; that portion of the branch distal to the canker was dead.

Jan. 9 (1917). Several *Monilia* pustules present on the dead spur.

Variety *Lord Derby.*

On this variety the inoculations which resulted in wilting were made on trusses borne on short spurs at nodes on the main stem of a young tree. The flowering spurs were four in number and inserted at successive nodes, the stem being 0·6 to 0·7 cm. in thickness in this region. The uppermost truss (*a*), borne on a spur 2 cm. in length, was inoculated; at the next node below was an untreated control truss (*b*); the third (*c*), on a spur 1 cm. long, was inoculated while the fourth (*d*) was another control. Within a few centimetres of (*a*) the stem divided into three branches all bearing leaves only.

April 19. The first flower to open (i.e. in the centre of the inflorescence) of each of the two trusses (*a*) and (*c*) was inoculated by placing conidia on the stigmas.

April 27. The styles of the inoculated flowers were withered and brown to the base and the stamens had collapsed. In the corresponding flowers (of the same age) on the control trusses the styles were not discoloured and the stamens were upright.

,, 29. All the flowers of the inoculated trusses were drooping and the leaves were beginning to wilt; the stamens of the untreated flowers were however rigid (not collapsed with distorted filaments as in the two inoculated flowers).

May 1. All the leaves of trusses (*a*) and (*c*) were by this time curled, brown and withered: the stamens of the non-inoculated flowers were still rigid. (This stage is shown in Figs. 3 and 5.)

,, 5. From truss (*c*) the disease had traversed the tissues of the spur and was invading the main stem as shown by a slight sinking of the bark at the base of the spur and extending about half the way round each side; truss (*b*) was just beginning to wilt, indicating that the transpiration current was being interrupted, and that the vessels of the xylem were attacked.

,, 10. The canker at truss (*c*) had almost girdled the stem and extended 0·5 cm. upwards and 1·5 cm. downwards from the base of the spur; the cankered area was indicated by a distinct wrinkling of the bark which was also slightly depressed below the general level.

,, 11. The stem was now completely girdled; the lowest branch distal to the affected spurs began to wilt.

,, 19. The leaves of the other two branches above the spur were drooping and turning brown.

June 2. A canker had also developed from truss (*a*) so that by this date the two cankers had united and together they extended along the stem for a distance of 10 cm., i.e. from 4 cm. above (*a*) to 6 cm. below (*c*); truss (*d*) however was still alive. Towards the upper end of the canker there was a series of dark lines more or less parallel to one another giving a zonate appearance. That portion of the tree above the infected spurs was by this time quite dead. (This stage is shown in Figs. 4 and 6.)

Dec. 6. Young pustules were bursting through the bark at the lower end of the canker; the immature conidia remained cohering in chains when mounted in water.

Jan. 9 (1917). Numerous conspicuous pustules were present along the canker and on the infected spurs; the conidia were more or less pulverulent and a considerable number became free on mounting in water. Such conidia were viable for many of them germinated within 48 hours in distilled water at a temperature of 6° to 8° C.

(*b*) *Inoculation of apple flowers in the Plantation.*

The experiments carried out in the greenhouse furnished definite proof that the fungus is a true parasite under favourable circumstances. It was desirable however that similar experiments should be performed in the plantation in order to obtain evidence that the disease could be induced in a similar manner on trees growing in the open.

Early in May 1916 inoculations of apple flowers in the plantation were made for comparison with the results obtained under glass. The flowers of the variety Warner's King began to open about May 1 and as this variety was known to be susceptible to the disease it was selected for the experiments.

As before, the conidia were obtained from cultures, about a week old, growing on steamed potato, but the method adopted in the actual inoculation of the blossoms was slightly modified, in order to avoid any injury to the stigmas that might occur when using the platinum wire in transferring the conidia from the cultures. The steamed potato, with the fungus, was removed from the culture tubes (using sterilised instruments) and placed in sterilised petri dishes; small portions bearing tufts of conidiophores were cut off with flamed scalpels and placed in another sterile petri dish which was taken direct to the plantation. In each case only one flower of each truss was inoculated and the inoculation was made by removing from the dish, on the point of a sterile needle, a particle of potato bearing conidiophores and bringing the conidia in direct contact with the stigmatic surfaces, so that the needle itself in no case touched the stigmas. The selected flower of the truss was marked, for identification and comparison with the rest, by a few inches of white cotton tied loosely round the flower.

Variety *Warner's King*.

Results of inoculations:

(*a*) May 5. One flower of a truss inoculated on the stigmas.
,, 11. One style of the inoculated flower brown to the base.
,, 18. The stamens of this flower had collapsed, while those of

other flowers of the inflorescence were upright. On this date the treated flower was accidentally removed during the examination and the disease made no further progress, the spur remaining alive throughout the summer.

(*b*) May 5. One flower inoculated, borne on a spur 2 cm. in length.
,, 11. One style brown in the middle.
,, 18. Stamens collapsed (stamens of the other flowers of that truss upright).
,, 20. Flowers and leaves of the truss wilting.
,, 29. The bark round the base of the spur beginning to crack.
Oct. 19. The disease had not extended further; the spur was dead to the base and the bark on the branch was fissured just below the insertion of the spur but there was no definite depressed cankered area.
Jan. 10 (1917). Conspicuous *Monilia* pustules present on the dead spur.

(*c*) May 5. One flower inoculated, on a branched spur bearing two trusses.
,, 11. Two styles brown to the base, two others brown for about half their length.
,, 17. The leaves round the truss wilting.
,, 18. *Monilia* pustules present on some of the dead pedicels.
Oct. 19. The infected half of the spur was dead to the base, a distance of 3 cm.; the rest of the spur, including the other truss, was alive.
Jan. 10 (1917). *Monilia* pustules present on the dead spur.

(*d*) May 5. One flower inoculated, on a branched spur bearing two trusses.
,, 18. Stamens of inoculated flower collapsed.
,, 20. Truss wilting.
Oct. 19. Infected half of the spur dead to the base with a canker two-thirds round the main axis of the spur; the rest of the spur was alive.
Jan. 10 (1917). *Monilia* pustules present on the dead portion.

(*e*) May 5. One flower inoculated, on a spur 5 cm. in length.
,, 22. Flower brown to the base of the pedicel.
,, 25. Leaves round the base of the inflorescence drooping.
,, 29. Truss quite withered, leaves brown.
Oct. 19. Spur dead for a distance of 5 cm.

Nov. 28. The spur bore several *Monilia* pustules with chains of conidia; the latter were not pulverulent and evidently immature.

Jan. 10 (1917). Numerous conspicuous pustules present along the whole length of the spur; conidia more or less pulverulent (a large number becoming free on mounting particles of the pustules in water).

Although the conidia were obtained when the temperature of the air was below 0° C. many of them germinated within 48 hours, in a drop of water kept at 6° to 8° C.

(*f*) May 5. One flower inoculated of an unbranched spur 2 cm. in length.

,, 16. Flower discoloured to the base of the pedicel.

,, 18. Leaves at the base of the truss drooping.

June 2. Bark on the branch cracking immediately above and below the insertion of the spur; a slightly depressed area extended one-quarter round the branch.

,, 26. Canker now extended half round the branch.

Oct. 19. The canker had not extended any further round the branch; it was however quite well defined but at this date bore no *Monilia* pustules.

Dec. 1. Immature pustules (conidia not pulverulent) present along the whole length of the spur and on the canker.

Jan. 10 (1917). Pustules more conspicuous: conidia more or less pulverulent; many germinated in distilled water within 48 hours at 6° to 8° C.

A flower on each of three other trusses was also inoculated; the three flowers died but became detached about May 20 and the disease did not extend into the axis of the inflorescence. The inoculation of three flowers on May 10 and five on May 11 did not produce any wilting of the trusses and it may be that in these cases the flowers were too old for successful infection, although at the time of inoculation the stigmas appeared receptive and showed no discoloration. These results correspond to those obtained in the greenhouse where, as already shown, the inoculations were most successful on those flowers which had recently expanded.

It will be observed that in every case where inoculation with the fungus was followed by the death of the truss, *Monilia* pustules appeared on the cankers and dead spurs during the succeeding winter. Usually

they began to burst through the bark during December and many well-developed pustules with viable pulverulent conidia were to be seen by the middle of January.

Although it has been noticed that in some cases spurs showing the typical symptoms of the Blossom Wilt in the summer failed to develop *Monilia* pustules in the winter, this condition is exceptional, and where cankers have been formed these invariably have produced the conidial stage of the fungus. The results of the artificial inoculations too demonstrate that a spur showing the wilt condition in summer is almost certain to produce pustules of conidia before the following spring. The danger of allowing such spurs and cankers to remain on the trees until the next flowering season is obvious.

(*c*) *Inoculation of Twigs through Wounds.*

Twigs on trees of the Newton Wonder variety, which is known to be susceptible, were inoculated with mycelium from a plate culture of the same strain which produced wilting of the blossom in the inoculations made on the Warner's King variety. A Λ-shaped cut was made through the bark, the triangular portion turned back, and agar bearing vigorously growing mycelium was placed between the wood and the bark; the latter was then gently pressed back and the wound covered with sterile tinfoil which was secured in place by means of raffia. Three wounds were treated in this way and three others, as controls, were not inoculated. The inoculations were made on May 30, that is, at the time when cankers were in process of development on trees infected through the flowers. When examined some months later all the wounds were covered with callus and no trace of canker was to be found on any of them.

The result suggests that the fungus does not readily (if at all) produce cankers by infection through wounds on the branches, and agrees with observations in the open where, so far as my own experience goes, a canker produced on an apple tree by this *Monilia* invariably originates in a spur that has been infected through the flowers.

(*d*) *Inoculation Experiments on the Fruit.*

It has already been pointed out that the *Monilia* which causes the Blossom Wilt may occur on the young apples. It will also grow readily on apples approaching maturity and on ripe apples after picking, as artificial inoculations have proved, although under natural conditions instances of its occurrence on the mature fruit appear to be rare for

the writer has not yet met with such cases even on trees seriously affected with Blossom Wilt.

Apples of the varieties Warner's King, Newton Wonder and Bramley's Seedling were inoculated on August 10 and 11 by placing mycelium from a plate culture in wounds made with a sterile scalpel. Unfortunately the apples became attacked by ants and all fell to the ground after a few days; the experiments however continued long enough to show that the fungus rapidly produced a brown rot appearing within four days as a discoloured area 1·5 to 3 cm. in diameter round each point of inoculation.

The experiments were then continued on apples of the Bramley's Seedling variety, which were brought into the laboratory and inoculated with a strain isolated from a canker. The first inoculations of this series were made on Aug. 17 and the experiments were repeated at intervals throughout September and October. For comparison, other apples were inoculated with *Monilia fructigena*, using a strain obtained from a plum, and with a hyaline form of *M. cinerea* also isolated from a plum. In some cases *M. fructigena* and the canker strain were placed in wounds made on opposite sides of the same apple, while in others the hyaline strain of *M. cinerea* and the canker form were grown on the same apple. Under these conditions it was found that each of the three forms produced a rot which extended approximately at the same rate for all, i.e. 2·5 to 3·5 from the point of inoculation in seven days. The canker form of *Monilia* however seldom produced pustules of conidia; on some of the fruit a few scattered tufts of white barren hyphae developed but on others no pustules were produced. On the other hand the strain of *M. fructigena* used in these experiments freely produced large yellow pustules more or less in concentric circles. The hyaline form of *M. cinerea* also developed conidia readily but on smaller grey pustules which were usually fairly numerous.

The skin of those apples inoculated with the canker *Monilia* rapidly assumed a dark brown shade over the affected area, which gradually became black. This nigrescence was to be detected towards the centre of the discoloured area about a week after inoculation; it gradually extended over the surface until the whole was black. The other two strains also produced some blackening particularly in the later experiments (i.e. on the more mature fruit) but not so readily. These differences were most striking in those cases where two forms were growing together on the same apple by inoculations at opposite sides. For example, two apples were inoculated on Aug. 17 and two others on

Aug. 21 by placing mycelium from an agar plate culture in wounds on opposite sides using the canker *Monilia* on one side and *M. fructigena* on the other. The result was the same for all four; that half of the apple infected with *M. fructigena* produced numerous large yellow pustules and remained brown, while the side infected with the canker form became quite black in from three to four weeks and developed no pustules at all or from one to three minute barren tufts of hyphae. The side inoculated with *M. fructigena* became shrunken at a much more rapid rate than the other.

Similar experiments were performed about the same time using the canker-producing *Monilia* and the hyaline *M. cinerea* strain. Again that side of the apple infected with the former soon became black and bore few or no pustules, while the opposite side produced, as a rule, numerous greyish pustules and remained brown for some weeks, becoming however gradually darker until it was almost black; as before the shrinking of the skin was most pronounced on the pustular side.

Later other apples of the same variety were inoculated using the same three strains of *Monilia* but infecting each apple with but one of the three. The results conformed with those obtained previously; those apples infected with *M. fructigena* became almost covered with large yellow pustules often becoming confluent, those with the hyaline strain of *M. cinerea* produced smaller greyish pustules, while those with the canker form remained sterile or produced a few scattered tufts of aerial mycelium usually sterile.

The apples used in these experiments were such as showed no apparent injury before inoculation; they were obtained from trees growing in the College plantation and taken immediately to the laboratory and inoculated. As the crop was picked during the first week of October subsequent inoculations were made on apples (of the same variety) from the fruit-storage shed. On such fruit the results were practically as before except that the barren hyphal tufts produced by the canker-strain were rather more numerous than in previous experiments. This was probably due to small abrasions caused during the operations of picking and storing or to minute cracks produced in the skin on drying, thus allowing hyphae to grow out into the air. But even then the difference between these results and those obtained with the other two forms of *Monilia* was still conspicuous.

Whether similar results are to be obtained with other varieties of apples has not yet been determined but it appears evident that the *Monilia* causing the blossom wilt and canker of apple trees produces

conidia on the ripening fruit much less readily than *M. fructigena*, and this probably accounts for its inability to establish itself on the mature apples, for the chief sources of infection from this fungus (i.e. the pustules on the cankers and spurs killed in the previous season) have shed most of their conidia and are becoming desiccated at the time when the apples are reaching maturity. As indicated earlier in this paper the newly formed cankers do not produce conidia until long after the fruit is picked.

The blackening of the skin of apples produced by the blossom wilt fungus may also be caused by *M. fructigena* and by the hyaline grey *Monilia*, particularly on the mature fruit, but the result takes place much more gradually. Black apples with few or no pustules are frequently found among stored fruit; this condition appears to be brought about by *M. fructigena* which has invariably been isolated by the writer from such apples obtained from fruit growers in Kent.

Spinks (22) finds that a similar "Black Rot" of cider apples is also to be attributed to *M. fructigena*.

VI. Control Measures.

It is evident that cutting away the dead spurs and cankers removes the chief source of infection, and an experiment, carried out on the row of trees of the Warner's King variety referred to in preceding pages, has demonstrated that where this can be done thoroughly the results are highly satisfactory.

Ten trees at one end of the row were carefully pruned by the writer on June 15 and 17, 1915, and so far as could be seen at the time every withered truss was cut away until all the dead discoloured (brown) tissues of the spur were removed. In those cases where cankers had developed on the branches the operation involved the removal of dead bark and wood in those places, until a clean cut, showing healthy tissues only was made. The trees were not treated in any other way and the wounds made by the pruning knife were left exposed. In all over 220 dead spurs were removed from these trees. When these ten trees were examined in the summer of 1916 it was found that five were quite free from the disease, one tree had but one dead truss, three had two each, and one had six dead trusses; a search on the last mentioned tree however revealed the fact that two dead spurs had been overlooked during the pruning operations and these now bore a number of *Monilia* pustules.

The rest of the trees (fourteen in number) in the same row had been

pruned in autumn in the usual way and no special instructions with regard to the disease has been issued to the men entrusted with the work. The consequence was that although a certain amount of "dead wood" had been cut away a considerable number of spurs and cankers, which subsequently produced the conidial stage of the fungus, remained on the trees. In some cases a dead spur had been cut off close to the branch but the diseased tissues of the canker round the base had been left and later the fungus appeared there. In the spring of 1916 it was found that every tree bore the fungus in its infectious (conidial) condition, the number of dead spurs and cankers with pustules varying from one to twenty-five per tree with an average of eight. The number of wilting trusses on these trees in May 1916 was 159 and varied from two to twenty-nine per tree; these numbers would probably have been considerably higher but for the fact that the trees had produced that year exceptionally few trusses of bloom. On several trees however more than one-fifth of the trusses wilted and in one case more than half of those present were killed.

Thus on those trees from which all dead spurs had been removed (omitting the one on which two had been overlooked) the number of wilted trusses was reduced, on the average, to less than one per tree, the infection in these cases being doubtless due to air-borne conidia from the infected trees in the rest of the row, while those trees on which infected spurs were left had an average of eleven wilted trusses per tree.

The ideal mode of treatment would be the removal of diseased trusses immediately after they first show signs of wilting; this would involve examining the trees three or four times at intervals of about a week between one examination and the next, all wilting trusses being removed and the spurs cut back until all brown bark and wood is removed, the first of these operations to be done about fourteen days after the earliest flowers open. This method would prevent the development of cankers which are not only much more troublesome to remove than the spurs themselves but it would prevent the girdling and death of the small branches.

As such measures would usually be impracticable except in small plantations of bush trees where the disease has not yet become rampant, an alternative would be to prune off all diseased spurs and cut out the cankers as occasion permitted during the summer. This work should be done as early as possible for the dead trusses with their withered brown leaves are easily distinguished from the healthy ones and clearly

indicate where pruning is necessary. If the operation is left over until the winter or spring very careful search is necessary to avoid overlooking some of the dead spurs and cankers, although even in winter the diseased spurs are often easily recognised from the fact that at leaf-fall the leaves and remains of flowers on such spurs still remain on the trees, and the pedicels and petioles may still be found on them in the spring.

When, owing to difficulty in obtaining the necessary labour, the removal of the spurs and cankers cannot be carried out before leaf-fall it may be done any time during the winter, but it is imperative that all diseased parts be cut off before any of the flowers begin to open.

It is important to emphasise the fact that the operation whenever carried out must involve the removal of all brown and dead wood and bark. Thus it is not sufficient merely to break off the withered trusses. In one experiment six withered trusses were broken off from their spurs early in June 1915, at the base of that season's growth, the spurs being then labelled for future reference; in April 1916 four of these bore *Monilia* pustules.

As already shown the disease occasionally appears on the young apples causing them to become dry and withered, and such "mummified" apples may be retained on the trees until the following year. Since it is impossible to distinguish, without close examination, between this form of Brown Rot and *Monilia fructigena* when occurring in this way it is necessary that all "mummies" be picked and destroyed during the winter, especially as *Monilia fructigena* itself is the cause of a serious fruit rot.

The cutting out of all affected parts before the blossoms open is the only treatment that can be recommended with confidence until further investigation is carried out. Owners of large standard Lord Derby apple trees have in some cases found, however, that to cut out all the dead trusses (which in serious attacks often number some hundreds per tree) is not practicable especially when skilled labour is difficult to obtain[1]. In those cases where the disease is very severe, "top-grafting" with a less susceptible variety is to be recommended.

Whether spraying is of value in controlling the disease is at present uncertain. As infection takes place through the open flower the use

[1] Our Sussex correspondent writes: "To cut off and burn the millions of diseased spurs and shoots on apples and plums is quite impracticable here."

"Millions" is probably no exaggeration in the case of large orchards, for the writer has counted over 130 wilted trusses (or about one-third of the whole) on a small bush tree, and has seen large standard trees where the proportion of dead to living trusses was even higher.

of a "cover-spray" to protect the stigmatic surface is out of the question, therefore a wash to be effective must be applied before the flowers open and must be capable of destroying the powdery conidial stage, or at least must prevent the conidia from falling during the period throughout which the flowers are open and receptive.

Experiments have been tried in the open with the Lime-sulphur Wash, which is frequently recommended for cases of Brown Rot, but no favourable results have been obtained. "Winter-washing" with Lime-sulphur failed to check a serious outbreak of the disease in the following spring. Facilities for testing the value of Lime-sulphur as a summer spray were kindly offered by Mr P. Manwaring[1] of Horsmonden, Kent, who permitted a plantation of Lord Derby trees (four rows each with thirty-two trees), which had had a severe attack of the Blossom Wilt in the previous year, to be used for experimental purposes. Three rows were thoroughly sprayed with Lime-sulphur at "summer strength" (S.G. 1·01) immediately before the flowers opened, the fourth row remaining untreated and kept as control. In May all the rows showed a serious attack of the Blossom Wilt and no difference in intensity could be detected between the unsprayed row and the rest; many of the latter had 50 % or more of the trusses killed by the fungus, and the numerous *Monilia* pustules on the dead spurs were apparently uninjured by the spray fluid.

This result is doubtless due to the fact that such a liquid as the Lime-sulphur solution is unable not only to penetrate but to adhere to the powdery pustules. Experiments carried out in the laboratory showed that Lime-sulphur solution even when applied in the form of a very fine spray with an atomizer immediately ran off from the pustules which appeared totally unaffected by the treatment.

Bordeaux Mixture applied similarly with an atomizer adhered a little more readily but the majority of the pustules were not covered by the spray.

An Ammonium Sulphide solution containing soft-soap, as recommended by Dr Eyre and Mr Salmon[2] for use on the conidial stage of the *Erysiphaceae*, was also tried in the same way. This wash did wet the pustules which in consequence became brown on the surface and lost their pulverulent appearance. The pustules themselves were not

[1] I take this opportunity of thanking Mr Manwaring for the facilities offered for investigating the disease in his plantations.

[2] Eyre, J. Vargas and Salmon, E. S. The Fungicidal Properties of Certain Spray Fluids. *Journ. Agric. Science*, Vol. VII. pp. 473-507.

killed and conidia placed in hanging drops germinated readily. It would seem however that the surface layers were killed and the question is whether this would be sufficient to prevent the fall of the conidia during the critical period when the flowers are open if the spraying were done as late as possible but before the flowers expanded. It is proposed to carry out experiments in the open to test this point.

In conclusion I desire to thank Prof. L. R. Jones (Wisconsin), Dr G. B. Posey (Oregon), Mr McCubbin, M.A. (Ontario), and Dr Quanjer (Holland), who have kindly sent specimens of mummified fruit or cultures from abroad, also Dr Pethybridge and Mr J. M. Hector, B.Sc., who sent mummified fruit and diseased branches. I am indebted also to Mr Salmon (head of the Mycological Department at Wye College) whose advice and criticisms throughout the investigation have been invaluable.

Summary.

1. A "Blossom Wilt and Canker" of apple trees, produced by a species of *Monilia*, is causing great loss to fruit growers in the south-east of England.

2. Infection takes place through the open flowers; the fungus invades the tissues of the flowering spur, thus killing the inflorescence and the leaves of the spur; the disease may reach the branch and produce a canker.

3. Spurs killed during the summer, together with the accompanying cankers, produce pustules of conidia during the following winter and spring; these conidia, falling on the flowers as they open, give rise to a new outbreak of the blossom-wilt disease.

4. When a canker has shed its crop of conidia it becomes covered with callus which eventually heals the lesion.

5. Inoculation of apple flowers with conidia from pure cultures of the fungus resulted in the death of the inflorescences and the spurs; in some cases cankers were produced. Conidia-bearing pustules of the organism appeared on these dead spurs and cankers during the following winter.

6. The causal organism is a grey *Monilia* easily distinguished from *M. fructigena*; at present it is to be referred to *Monilia cinerea* Bon.

7. On culture media the habit of the fungus is different from that of the grey *Monilia* (also referred to *M. cinerea* by American workers) which is commonly found in North America.

8. The disease may be kept in check by cutting out all dead spurs and cankers before the flowers open; on the first appearance of the disease all wilted trusses and dead spurs should be promptly removed. Spraying operations can be efficacious only when they kill the conidial pustules or prevent them from shedding their conidia during the flowering period.

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DESCRIPTION OF PLATES XXII—XXIV.

Fig. 1. Portion of cankered branch (var. Lord Derby) showing *Monilia* pustules; condition as seen in winter. (Photographed April 8, 1916.)

Fig. 2. Dead spur and canker bearing *Monilia* pustules, with neighbouring flowering spurs; the wilted trusses below the canker were probably infected by conidia falling from the spur and canker; condition as seen in summer. (June 4, 1915.)

Fig. 3. Four trusses on the main stem of a young Lord Derby apple tree; the first and the third (from above) were each inoculated, from a pure culture of the fungus, on a single flower. Result fifteen days after inoculation—both inoculated trusses are dead.

Fig. 4. As in Fig. 3 but four weeks later; the stem is cankered above and below the two inoculated trusses.

Fig. 5. The stem of the infected tree at the time that the photograph shown in Fig. 3 was taken; the four trusses there shown are to be seen immediately below the branch on the right.

Fig. 6. As in Fig. 5 but taken four weeks later, i.e. on the same day as Fig. 4 was obtained. The leaves on the branches above the canker are wilting.

Fig. 7. A canker produced by a natural infection in 1914; it bore *Monilia* pustules in 1915, but when photographed in June 1916 was barren and was being covered over by callus.

Fig. 8. The canker shown in Fig. 2 seen in transverse section (× 2½). The development of callus at the sides of the canker has already commenced at this stage.

(Figs. 1—4 and 7 are ⅝ natural size.)

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Fig. 2

Fig. 1

Fig. 4

Fig. 3

Fig. 5

Fig. 6

Fig. 7

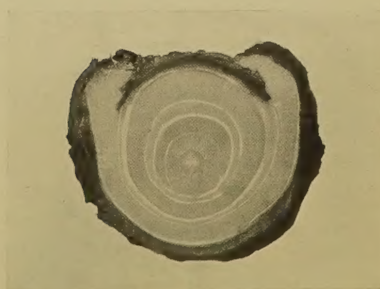

Fig. 8